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GENETIC CHANGES IN POPULATIONS UNDER IRRADIATION[1]

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The precipitate advent of the atomic age has evoked a deluge of articles concerning the biological harm resulting from radiations; geneticists, physicists, and medical scientists have all contributed their views. While the chief concern has been the immediate physiological effects of the radiation, there has been a tendency to speculate on the long-range effects which genetic changes induced by radiation will have on human populations. These speculations have ranged from badly reasoned, alarming statements in popular magazines to impressive (although not necessarily less alarming) arguments marshalled by competent scientists, including Haldane (1947), Muller (1948, 1950, a, b, and c), Evans (1949), and Wright (1950). Although these arguments are well organized, the divergent conclusions emphasize two points: the need for more knowledge, and, until that is available, the need for caution in exposing individuals to radiation.

In general, two classes of data may be gathered to further our knowledge of the genetic effects of radiation on populations. Analyses can be made of separate gene loci in order to determine mutation rates, degrees of semidominance, relative frequencies of mutant alleles with differing degrees of subvitality, the time of action of these deleterious alleles, and the role radiation plays in altering these factors. Using this knowledge, and the mathematics of population genetics, equilibria could be predicted for various classes of mutant genes and the relative importance of these classes in the total effect exerted by mutant genes on the well-being or adaptive value of a population.

A second general approach to the problem of irradiated populations, the one discussed in this paper, is the study of the population itself. The drawbacks of this method are that individual mutations are not followed, that the data reflect mass changes within the population, and that analyses can be made only on simplified assumptions. Nevertheless, the advantages of this approach are several: 1. The information obtained is what is wanted for practical purposes. 2. This information must be available for evaluating the results obtained by substituting figures for individual gene mutation data in the formulas of theoretical population genetics. (That the use of these general formulae will not be simple is indicated by this statement of

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Wright's (1932, p. 155): "...the selection coefficient of a particular gene is really a function not only of the relative frequencies and momentary selection coefficients of its different allelomorphs but also of the entire system of frequencies and selection coefficients of non-allelomorphs.") 3. Data obtained from populations may disclose evolutionary changes evoked in response to radiation; these changes cannot be considered in the gene mutation studies, for the latter are essentially static.

The purpose of the present paper is to outline the basic postulates of the research project, to present typical data, and to discuss some of the implications of these data. The assistance of Carol V. Madden, Louis Forgione, Howard Monsees, Gloria Cosillo and Henry Gardner is gratefully acknowledged. Frequent discussions with Professor Th. Dobzhansky of Columbia University have been a continual source of inspiration. Drs. A. E. Brandt and Howard Levene of the U. S. Atomic Energy Commission and Columbia University, respectively, have given helpful suggestions concerning the handling of statistical problems.

MATERIAL AND METHODS

Six populations of *Drosophila melanogaster* were studied in order to determine the effect of radiation on populations. The analyses were concerned primarily with the second chromosome, one of the two large autosomes of this species. The original parental flies of each population carried second chromosomes that were free of lethals, semilethals, and easily detectable subvitals.

The breeding system used to test individual chromosomes in the homozygous condition is illustrated in fig. 1. Egg samples were taken from the populations at two-week intervals (or multiples thereof) and were subdivided among several cultures in order to minimize larval competition. Males (P_1) which hatched from these eggs were mated individually with *CyL/Pm* virgin females. (*CyL* designates a balancer chromosome, *Cy* al^2 lt^3 L^4 sp^2, that carries a crossover suppressor in each limb and the two dominant genes *Curly* and *Lobe*. *Pm* refers to another second chromosome marked with the dominant gene *Plum* and which is, in turn, a convenient balancer for *CyL*.) One *CyL/+* (F_1) male was picked from each P_1 culture and was remated with *CyL/Pm* females. The *CyL/+* male and female (F_2) offspring of this mating carried identical wild-type second chromosomes and were inbred to obtain an F_3 which gave, if the tested chromosome carried no deleterious genes, *CyL/+* and *+/+* flies in a ratio of approximately 2:1. If the tested chromosome carried a lethal, only *CyL/+* flies appeared in the F_3 culture; if the tested chromosome carried a semilethal, the 2:1 ratio was distorted in favor of *CyL/+*. Since genes that affect viability do not fall into sharply defined classes, we adopted the following conventions: a lethal was defined as a chromosome which permitted 0%–3.1% homozygous wild type (non Curly-Lobe) individuals to emerge in an F_3 culture; a semilethal, 3.2%–15.8%; and a "normal," 15.9% or more. If a culture contained 50% or more wild-type flies, it was discarded; this happened very rarely.

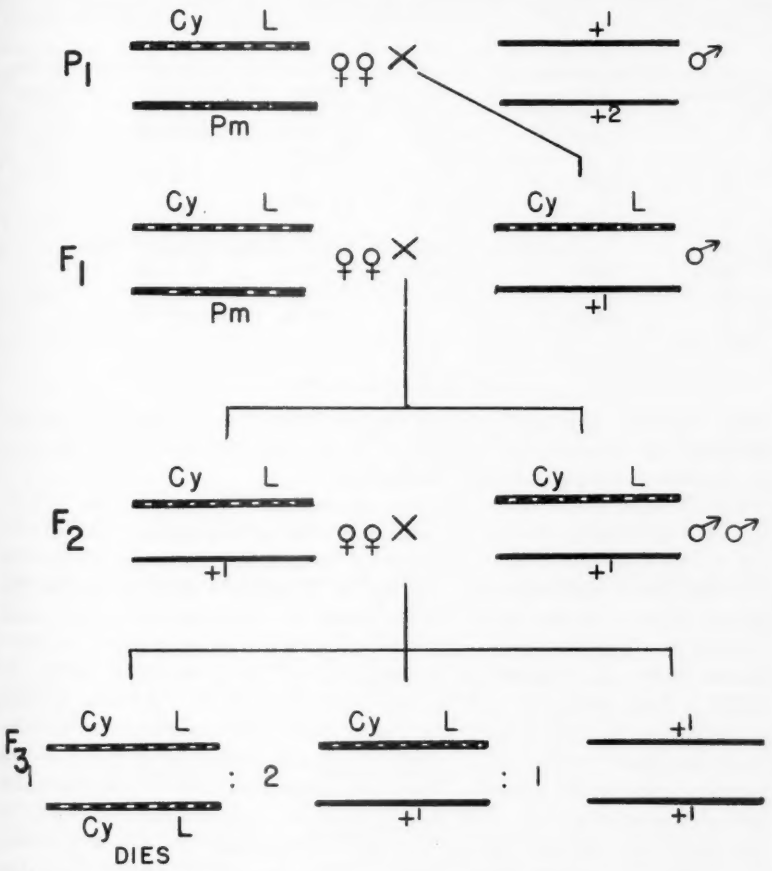

FIGURE 1. Mating system for the detection of recessive, second-chromosome genes.

Summarized descriptions of the populations and the experimental conditions are given in table 1. All populations were kept at 25°C. The sources of chronic gamma radiation were two radium bombs approximately 70 mg and 500 mg in size. All populations except #5 were maintained on cornmeal-molasses-agar medium enriched with brewer's yeast; these populations consisted of approximately 10,000 adults. Population 5 was a small population of 100–1000 adults that was maintained on un-enriched medium. The populations exposed to chronic gamma-radiation were kept in semi-circular lucite and screen population cages; these cages have been described previously (Wallace, 1949). The populations not exposed to chronic radiation were kept in oblong lucite and screen cages 18″ long, × 5½″ wide, × 4½″ high.

TABLE 1

DETAILS OF THE EXPERIMENTAL POPULATIONS

Population	Size	Treatment	Dose	Started	Stopped
1	Large	Acute	♂♂ 7000r ♀♀ 1000r	7/25/49	...
2	Large	Acute	♂♂ 1000r ♀♀ 1000r	7/25/49	1/12/50
3	Large	None	...	7/25/49	...
4	....Special population for determining mutation rates....				
5	Small	Chronic	5.1r/hr	4/ 1/50	...
6	Large	Chronic	5.1r/hr	4/15/50	...
7	Large	Chronic	.9r/hr	4/15/50	...

EXPERIMENTAL RESULTS

The data that were obtained from the populations were of two types—those revealing the chromosomal content of the populations and those indicating the condition of the populations themselves.

The changes in frequency of lethal chromosomes in populations 1, 3, 5, 6, and 7 (population 2 was discontinued after 12 generations and will be left out of this discussion) are given in table 2 and shown graphically for the four latter populations in fig. 2. It is obvious that, except for the initial decrease in the frequency of lethals in population 1, lethals accumulated in all populations. The rates of accumulation in the various populations were: (1, samples 4–34) 0.45% ± 0.07%, (3) 0.38% ± 0.05%, (5) 3.88% ± 0.5%, (6) 5.36% ± 0.5%, and (7) 1.04% ± 0.2%. The rate of accumulation in #3 agreed with previously determined spontaneous mutation rates for this chromosome (0.4%–0.5%) if one recalls that the two-week sample interval was probably somewhat shorter than the actual generation time in the populations. Even though the rate of accumulation of lethals in the chronically exposed populations need not represent the rate of mutation, it is interesting to note that exposure to 300 r per sample interval more than doubled the rate at which lethals accumulated and that the ratio between the rates of accumulation in populations 6 and 7 was approximately proportional to treatment. Lethals accumulated in populations 5 and 6 at quite similar rates—the difference in the calculated figures resulted primarily from the last sample of 5. It is impossible to tell at the present time whether this divergent sample from 5 represented genetic drift within a small population or the approach to equilibrium in a small population at a low frequency of lethals. Finally, it may be mentioned that the rates of accumulation in populations 1 and 3 were nearly identical following the initial elimination of lethals from population 1; the lethals eliminated quickly were probably those associated with translocations (Wallace, 1951).

Semilethals (table 3) are much rarer as a class than lethals; their accumulation in the control population and in population 7 was exceedingly slow (0.09 ± 0.015% and 0.05 ± 0.03%). Following the original x-radiation of population 1 semilethals were present and showed a subsequent increase

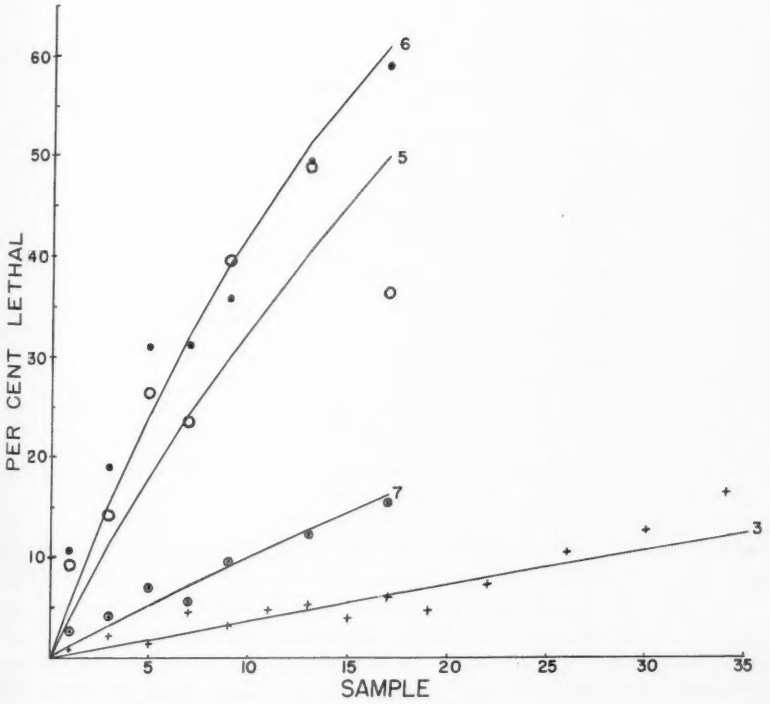

FIGURE 2. Frequencies of lethals recovered in samples of chromosomes taken from experimental populations; +, population 3; ○, population 5; ●, population 6; ⊙, population 7. Theoretical lines fitted to the observed frequencies are identified by number.

TABLE 2

FREQUENCIES OF LETHALS IN SAMPLES OF CHROMOSOMES TAKEN FROM EXPERIMENTAL POPULATIONS OF *D. MELANOGASTER*

Sample*	#1	#3	#5	#6	#7
1	18.3%	0.8%	9.1%	10.7%	2.7%
2	14.1	0	...	...	...
3	11.6	2.2	14.2	19.0	4.2
4	10.1	...	...	...	...
5	...	1.4	26.4	31.0	7.0
6	10.9	...	...	...	...
7	...	4.6	23.6	31.3	5.8
8	13.7	...	...	...	...
9	...	3.2	39.4	35.7	9.6
10	14.6	...	...	...	...
11	...	4.9	...	...	...
12	14.5	...	...	...	...
13	...	5.2	48.9	49.2	12.2
14	13.6	...	...	...	...
15	...	4.0	...	...	...
16	18.8	...	...	...	...
17	...	6.1	36.2	58.8	15.5
18	18.7	...			
19	...	4.9			
20	13.8	...			
22	...	7.3			
24	20.0	...			
26	...	10.6			
28	25.4	...			
30	20.8	12.9			
34	24.2	16.5			

*Sample 1 of population 5 is really 20; of 6 and 7, 21.

exceeding that of population 3 (0.41 ± 0.07%; samples 1–20) and not differing significantly from those of populations 5 and 6 (0.51 ± 0.08%; 0.37 ± 0.07%).

Subvitals are genes with such small deleterious effects that they are difficult to demonstrate individually. Relevant data were obtained by computing the average frequency of wild type flies found in all non-lethal, non-semilethal test cultures of each sample. This average (table 4), obviously, would decrease if subvitals accumulate in the population. Unfortunately, since these data also reflect variations in culture conditions, only the data for populations 1 and 3 are sufficiently extensive to warrant discussion. The average viabilities of normal chromosomes from #1 were nearly always lower than those of population 3, their regressions (1. $Y = .3072 - .0002X$, $\sigma_b = .0002$; 3. $Y = .3172 - .0000X$, $\sigma_b = .0002$) have slopes not significantly different from O. (During the time from sample 1 to sample 20 there was an apparently significant decrease in the average viabilities of normal chromosomes of population 1 ($p = .02$–$.05$) but over the whole course of the experiment there is no significant change.)

An estimation of the effects of radiation on populations can be obtained by selecting a character of presumed adaptive significance and making com-

TABLE 3

FREQUENCIES OF SEMILETHALS IN SAMPLES OF CHROMOSOMES TAKEN FROM EXPERIMENTAL POPULATIONS OF *D. MELANOGASTER*

Sample	#1	#3	#5	#6	#7
1	3.7	0	2.4	1.2	1.6
2	3.7	0	...	...	...
3	4.6	0	4.5	3.3	1.2
4	1.9	...	...	...	...
5	...	0	3.8	3.4	1.3
6	5.6	...	...	...	...
7	...	1.2	7.4	5.9	2.3
8	4.7	...	...	...	...
9	...	0.4	5.5	3.7	1.1
10	6.2	...	...	...	...
11	...	0.7	...	...	...
12	8.5	...	...	...	...
13	...	1.1	8.7	6.4	2.2
14	8.2	...	...	...	...
15	...	2.2	...	...	...
16	10.7	...	...	...	...
17	...	2.1	11.2	8.0	2.2
18	11.9	...			
19	...	1.5			
20	8.0	...			
22	...	1.9			
24	9.1	...			
26	...	1.5			
28	0	...			
30	6.2	1.7			
34	7.9	3.8			

parative studies of this trait in irradiated and non-irradiated populations. An ideal study would include all factors involved in the perpetuation of the population and would lead to a determination of the adaptive value obtaining under the experimental conditions. A complete study of this type is impractical for a number of reasons and so alternate methods must be used.

The method we chose for preliminary studies consisted of a modified series of routine test crosses. A typical individual in a population is the product of random mating and, consequently, carries chromosomes of different origins; the individual is heterozygous for many genes and, in all probability, its particular gene combination is unique. The method of reconstituting individuals representative of those found in a population is shown in fig. 3. At the time the F_2 flies were collected in the routine crosses, no information was available concerning the nature of the wild-type chromosome; this information was available only when the F_3 culture had been examined. In the modified test crosses (which may be referred to as "heterozygous crosses" in contradistinction to "homozygous crosses" of the standard test), F_2 flies from the test series were systematically outcrossed (males of a × females of b, males of b × females of c, males of c × females of d, ... males of n × females of a) as shown in fig. 2. Each

F_3 culture then contained *CyL/+* flies of two types and wild flies heterozygous for two second chromosomes that originally were carried by different males. Depending upon the frequency of lethals and the probability of their allelism in the population, these heterozygous crosses occasionally lead to an F_3 culture containing no wild-type flies. Generally, however, the two

TABLE 4

AVERAGE FREQUENCIES OF WILD-TYPE FLIES IN TEST CULTURES OF NONLETHAL NON-SEMILETHAL CHROMOSOMES TAKEN FROM EXPERIMENTAL POPULATIONS OF D. MELANOGASTER

Sample	#1	#3	#5	#6	#7
1	30.66%	32.08%	31.75%	31.43%	31.52%
2	31.94	31.65	...	...	...
3	31.11	31.46	30.77	30.60	31.41
4	30.39	...	...	...	...
5	...	31.49	31.39	30.55	30.81
6	30.79	...	...	...	...
7	...	32.14	30.36	30.35	31.27
8	29.63	...	...	...	...
9	...	31.64	30.31	30.99	31.42
10	29.85	...	...	...	...
11	...	31.40	...	...	...
12	30.81	...	...	...	...
13	...	31.79	30.11	28.11	30.60
14	29.99	...	...	...	...
15	...	30.84	...	...	...
16	29.90	...	...	...	...
17	...	30.87	29.07	28.87	31.64
18	29.26	...			
19	...	32.25			
20	30.33	...			
22	...	32.77			
24	30.63	...			
26	...	32.04			
28	31.04	...			
30	30.83	32.77			
34	29.72	30.19			

chromosomes carried no lethals or semilethals in common and the frequency of wild flies was approximately the expected 33.3 per cent. The average frequency of wild-type flies in the whole array of combinations was taken as an estimate of the adaptive value of the population; obviously, it represented the relative viabilities of different types of larvae and pupae competing in slightly overcrowded culture conditions.

The results of the heterozygous crosses are given in table 5. The interrelations of 3, 5, 6, and 7 were as one might expect; the chronic treatments resulted in lower estimated adaptive values and the effect varies with the treatment. Population 1, however, had distinctly more wild-type flies in the F_3 cultures than population 3. The relative adaptive values of the popu-

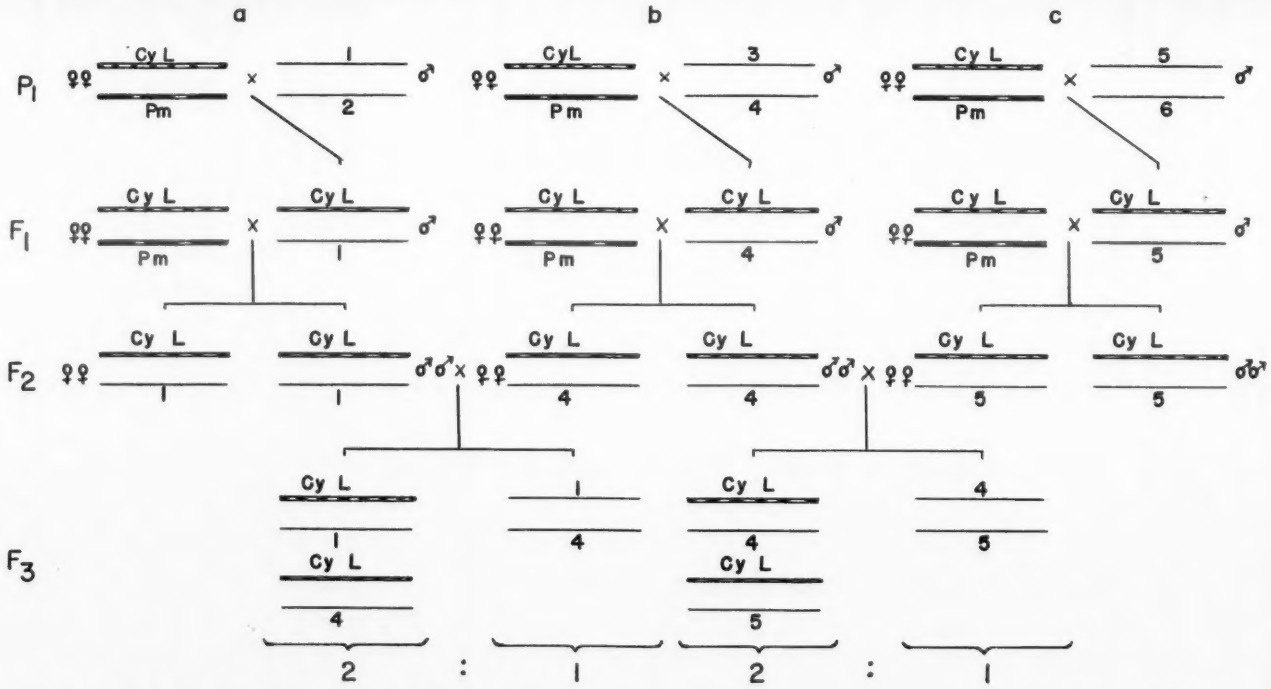

FIGURE 3. Mating system for studying viabilities of flies carrying second chromosomes of separate origin. Numbers and light lines represent wild-type chromosomes isolated from separate males.

TABLE 5

SUMMARY OF THE RESULTS OBTAINED FROM THE "HETEROZYGOUS" CROSSES DESCRIBED IN TEXT. (SEE FIG. 3)

Population	Sample	Number of combinations tested	% Lethal	% semi-lethal	Avg. freq. wild-type flies (%)	Estimated adaptive value of population
1	28	216	0	0	35.33	
1	32	312	0.3	0.3	34.19	
1	36	265	0	0.4	34.31	
1	40	201	0	0	36.90	
Total 1		994	0.1	0.2	35.02	1.04
3	32	274	0.4	0	33.12	
3	36	241	0	0	33.56	
3	40	207	0	0	34.82	
Total 3		722	0.1	0	33.75	1.00
5	30	163	0.6	0	31.48	
5	34	62	0	0	29.99	
5	38	165	0.6	0	31.06	
Total 5		390	0.5	0	31.07	.92
6	31	263	0.4	0	31.97	
6	35	209	0	0	31.74	
6	39	235	0.9	0	32.12	
Total 6		707	0.4	0	31.95	.95
7	31	256	0	0	32.91	
7	35	250	0	0.4	32.18	
7	39	224	0	0	33.48	
Total 7		730	0	0.1	32.83	.97

lations as revealed by this technique are: (1) 1.04 (3) 1.00 (5) .92 (6) .95 (7) .97.

DISCUSSION

The above account, while by no means exhaustive, describes and illustrates some of the more important types of data which can be extracted from experimental populations. Several questions concerning the evaluation and interpretation of the illustrative tables will now be discussed in more detail.

The first point that may be mentioned is the remarkable agreement between the rates of accumulation of lethals in populations 1 and 3 and the resultant constant difference of about 8 per cent in lethal frequency. This difference persisted for well over a year. The situation may be explained by assuming that the lethals which escaped elimination in population 1 during the first eight weeks (4 generations) were truly recessive and, because of the large number of lethal loci available (Wallace, 1950), were not appreciably eliminated by homozygosis. The situation can also be ex-

plained by assuming that the gradual elimination of radiation-induced lethals in population 1 was counterbalanced by a higher spontaneous rate of mutation of recessive lethals in that population. This explanation requires that the spontaneous rate of mutation to lethals changed in precise proportion to the change in the rate at which lethals were eliminated. In view of the long period over which the balance existed, it seems more likely that simple recessivity of lethals is the correct explanation. Proceeding from this premise, it is possible to compute the maximum degree of semidominance of lethals compatible with the data:

$$\frac{(b_3 + 1.3\,\sigma_{b_3}) - (b_1 - 1.3\,\sigma_{b_1})}{\text{average difference in lethal frequency}} = \frac{.0009}{.08} = 1\%$$

In the above expression, b_1 and b_3 are the slopes of the regressions of lethals in population 1 and 3, respectively; σ_{b_1} and σ_{b_3} are the errors of these slopes. The equation uses 1.3 σ in the ratio so that the confidence interval is nearer to the 95 per cent interval in general use; if 2 σ's are used, a 99.75 per cent interval is obtained and this is too large to permit a reasonably restrictive statement.

The data on average viabilities found in the different samples sheds light on the accumulation of subvital gene mutations in populations. If a population has initially only chromosomes which are normal when homozygous (average viability of 1), then the accumulation of subvitals of average selective disadvantage, $\bar{s}$, that arise by mutation at an average rate, $\bar{u}$, should lower the average viability by a factor $\bar{u}\bar{s}$ per generation. It may be postulated for convenience either that $\bar{u}$ is so low that most chromosomes carry only one subvital or that if a chromosome carries two subvitals, their combined effect when homozygous is additive.

The interesting populations in connection with subvitals are 1 and 3 because of their greater age and more numerous samples. The slope, b, of the regression is a function of $\bar{u}\bar{s}$; if there is no appreciable elimination of subvitals from the populations, $3b = \bar{u}\bar{s}$ (b is calculated in relation to an expected frequency of wild-type flies of approximately 33.3 per cent while $\bar{u}\bar{s}$ is given in relation to an average viability of 1). It is obvious that the product of $\bar{u}$ and $\bar{s}$ is small; in neither population 1 nor 3 has the slope differed significantly from 0. Because of the experimental variations, values of $\bar{u}\bar{s}$ equal to .0012 or .0018 would not be inconsistent with the data. The constant difference between the average viabilities of the two populations (as reflected by the similarity of the slopes of the regressions) argues against any considerable elimination of subvitals in the heterozygous condition. The data are not sufficiently precise to allow a decision regarding the initial increase in the average viability in population 1; the increase may be the result of sampling error or it may reflect the elimination of subvitals associated with translocations (these subvitals would be effectively semilethal in the heterozygous condition).

The most important characteristic of an irradiated population is its adaptive value. The purpose in studying rates of mutation to various types of

deleterious alleles at separate loci is that an estimate of the adaptive value can be made from a knowledge of the frequencies of homozygosity and the degree of disability of each deleterious homozygote. The result of such a study would be the comparison of an ideal adaptive value of 1 with calculated adaptive values of $1-s_1$, $1-s_2$, etc., where s is the decrease expected from the mutant genes.

The data obtained from the heterozygous crosses throw doubt on this simple procedure. For the sake of simplicity, consider populations 1, 3, and 7. The chromosomal data show that, at the time of the heterozygous crosses, population one had more lethals, more semilethals, and a lower average viability of flies homozygous for "normal" chromosomes than either 3 or 7 and that the two latter populations were quite similar in these respects. The occurrence of lethal and semilethal combinations in the heterozygous crosses indicated that the chance of two chromosomes of different origin having deleterious genes at the same locus was approximately the same in all three populations. However, when all tested combinations are considered, population 1 produced a higher frequency of wild-type flies than either 3 or 7 and 7 produced fewer than 3. The adaptive value, therefore, seems to be at least partially independent of observed frequencies of "deleterious" genes in the population.

The conclusion reached above depends, of course, on the technique of estimating the adaptive value. The most serious flaw in the method would arise if dominant subvitals were to distort the observed ratios of *CyL*/+: $^{+}$/+ flies by decreasing the frequency of *CyL*/+. We have noted, however, that the average viabilities of normal chromosomes in 1 and 3 did not converge; this indicates that subvitals in 1 were not semidominant. In the case of population 7 where genes were constantly mutating it was possible that some dominant subvitals were present, but this merely makes the calculated adaptive value of this population too high; in our argument we are regarding it as lower than that of population 3.

The results of the experiment have indicated, in brief, that while an examination of the individual chromosomes of a population may reveal that these are generally "deleterious" when homozygous, an examination of *pairs* of unrelated chromosomes from the same population may reveal that these pairs are distinctly superior. An insight into population dynamics is gained from this seeming contradiction. In a population (of Drosophila, for instance) where deaths are numerous between the fertilization of the eggs and attainment of sexual maturity, selection acts on combinations of genes; individuals survive that are carrying adaptively superior combinations. Meiosis and fertilization break up the selected combinations of each generation and make new ones from the available genes. The process is repeated again and again and the degree of "co-adaptation" (Dobzhansky, 1950) increases. The difference in the adaptive values of populations 1 and 3 can be explained on the basis of a larger number of potential combinations available in 1 than in 3. The currently higher adaptive value of population

1 could exist not merely *in spite of* but *because of* the original treatment. This may be a temporary affair; as more genetic diversity develops in 3, the adaptive values of the two populations will probably converge.

The low adaptive value of population 7 can be explained on the same basis but with one important facet remaining unknown. The basic selective process for superior combinations ("superior" in the population cage environment) could proceed in this population as in the others with a reshuffling of chromosomes occurring during meiosis and fertilization. The chromosomes that are reshuffled, however, are undergoing chronic irradiation and because of the mutations constantly occurring, are not the chromosomes that were originally chosen. This in itself is not a novel situation; spontaneous mutation leads to a similar condition in all populations. The precise reason why the adaptive value of 7 is lower than that of 1 or 3 is the important point: Has the number of generations that the population has existed been too few to allow for a comparable selecti on of heterosis? Does the amount of irradiation the population receives slow up the selective process substantially? Is selection inadequate to maintain superior combinations under these conditions? Time and additional experimentation will give the answers to some of these questions. The lower frequency of wild-type flies in populations 5 and 6 than in 7 indicate that chronic irradiation might disrupt coadaptation to a considerable degree. The data now available are not sufficient to allow the calculation of regressions of adaptive values. Other estimates of adaptive values are being obtained so that the data will not be limited solely to larval competition in F_3 culture bottles.

It must be re-emphasized in conclusion that the purpose of this paper was to introduce an experimental method of attacking a particular problem by presenting and discussing some typical data. The most important conclusions emerging at present are that heterotic combinations are selected for and become established in populations at an extremely rapid rate and that radiation-induced genetic variants are seemingly incorporated into these combinations. A corollary of practical importance is that, in estimating the genetic effects of radiations on populations, consideration must be given to the existence of coadapted genetic systems.

SUMMARY

A general account is given of studies made on experimental populations of *Drosophila melanogaster* which are exposed to radiations. A consideration of the frequencies of lethals, semilethals, and subvitals within the populations and of the estimates of the adaptive values of the populations suggests that heterotic gene combinations have been developed within some of these populations. The bearing these results have on estimation of radiation damage resulting from gene mutation is briefly mentioned.

LITERATURE CITED

Dobzhansky, Th., 1950, Genetics of natural populations. XIX. Origin of heterosis through natural selection in populations of *Drosophila pseudoobscura*. Genetics 35: 288–302.

Evans, R. D., 1949, Quantitative inferences concerning the genetic effects of radiation on human beings. Science 109: 299–304.

Haldane, J. B. S., 1947, The dysgenic effect of induced recessive mutations. Annals of Eugenics 14: 35–43.

Muller, H. J., 1948, Mutational prophylaxis. Bull. N. Y. Acad. Med. 24: 447–469.

1950a, Radiation damage to the genetic material. I. Effects manifested mainly in the descendants. II. Effects manifested mainly in the exposed individuals. American Scientist 38: 33–59; 399–425.

1950b, Our load of mutations. Amer. Jour. of Human Genetics 2: 111–176.

1950c, Some present problems in the genetic effects of radiation. Jour. Cell. and Comp. Phys., Vol. 35; Suppl. 1: 9–70.

Wallace, B., 1949, A new population cage. Dros. Inf. Serv. 23: 108–109.

1950, Allelism of second chromosome lethals in *D. melanogaster*. Proc. Nat. Acad. Sci. 36: 654–657.

1951, Genetic changes within populations after x-irradiation. Genetics (in press).

Wright, S., 1931, Evolution in mendelian populations. Genetics 16: 97–159.

1950, Discussion on population genetics and radiation. Jour. Cell. and Comp. Phys., Vol. 35; Suppl. 1: 187–210.

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DISCRIMINATION OF STREAM ODORS BY FISHES AND ITS RELATION TO PARENT STREAM BEHAVIOR[1]

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INTRODUCTION

The role of sensory mechanisms in orientation of fishes, especially in relation to homing, remains a mystery notwithstanding many efforts to solve this problem. It is the object of this paper to point up again the possibility that the sense of smell may play an important part in directing fishes to their home waters. The primitive character of this sensory system, its evolutionary constancy, its extreme sensitivity in comparison to other receptor processes, and its capacity to serve as a memory-evoking mechanism, all point to a working hypothesis—that olfactory stimuli may be factors in the homing of migrating fishes.

Scheer's (1939) review has summarized the results of the homing instinct in salmon, which he defines: "... salmon or trout hatched, and reared in a particular region will, upon returning to fresh water, return in the great majority of cases to the same region, even from considerable distances." While Scheer cited several workers whose marked fish had strayed from the home stream, one cannot fail to be impressed with the convincing data of Pritchard, Foerster and Clemens (1939) illustrating the accuracy of homing. Of 469,326 specimens of *Oncorhyncus nerka* marked before the oceanward migration, almost 11,000 returned to their parent stream, the Fraser River tributary of Cultus Lake. There was no straying. Allowing for heavy mortality at sea, these results of precise homing behavior defy explanation and point up the necessity for assuming a sense of great acuity.

While the idea has been mentioned by some writers (Scheuring, 1930, and Kyle, 1926) that homing in fishes may be ascribed to scent-perceiving tissues, details of such sensory control of migration have not been made clear. Craigie (1926) released 500 sockeye salmon, in half of which the olfactory nerves had been severed. Judging from later recaptures of the tagged normal and operated fishes, it was evident that the migratory behavior of the latter had been somewhat interfered with. White (1934) suggested that the fish oriented to milt shed into the water by precocious males; he presented no evidence on how it was detected.

[1]Supported in part by the Research Committee of the Graduate School from funds supplied by the Wisconsin Alumni Research Foundation and by the U. S. Office of Naval Research, Biological Sciences Division.

In the above observations, it could not be clearly proved that fishes orient on the basis of olfaction; nor has the idea been proposed that the home water carries a distinctive odor which through discrimination and memory may serve as a directing guide to the fishes in orienting toward and reaching its original habitat. The chief objective of our experiments was to determine whether streams do have characteristic odors and if fishes can discriminate between them, and whether they can retain in memory habits of orientation learned with respect to these odors.

It may be desirable, at this point, to define clearly the requirements of an odor which is to serve as a "sign-post" for returning salmon. First of all, it must remain relatively constant in any one stream over a period of years because an interval of three to five years may elapse between original learning of the olfactory controlled reaction and its reinstatement, upon return to the vicinity of the home water to spawn.

Since it is known that some salmon return to their home streams at the early age of three years, while others delay until four or five, it can easily be seen that the odor cannot be cyclical in nature, but must be present in the same form year in and year out. In this instance then, changes in the odor must not take place with more rapidity than evolution of the species, if the homing of the salmon is not to be disrupted.

The second condition which must be placed upon the odor is that it must have significance only to those returning migrants which had been conditioned to it during their freshwater sojourn; while being neutral to all others for it would seem that any odor, or substance, which was an attractant or repellent would induce salmon to enter a stream or tributary irrespective of whether or not they were native to the stream.

There is yet another restriction which must be placed upon a homing odor. It must remain detectable even though the stream be changed severely in chemical and physical characteristics, for salmon will continue to attempt to return to a stream even though that stream may have been seriously polluted, or gutted by floods, during the time the salmon were at sea.

It should be stated that the authors are not precluding the possibility that there may be other factors contributing to the ability of the salmon to return to their home waters. However, it is our opinion that of all the theories which have been suggested, that of a conditioned olfactory response is indeed plausible and seems to merit further exploration.

In this laboratory we (1949) demonstrated that natural odors, such as dilute rinses of aquatic plants, were perceived and discriminated by the bluntnose minnow (*Hyborhynchus notatus* Raf.). Moreover, these minnows were trained to respond to concentrations of phenol far below the odor threshold for man (Hasler and Wisby, 1950). Therefore, because our laboratory experience has been more extensive with the bluntnose minnow, it was adopted as the assay animal. Subsequently salmon were tested also.

METHODS AND MATERIALS

The water for this series of training tests was obtained from two creeks which drained watersheds of different edaphic conditions. One sample

was taken from Otter Creek at Meyer's Hill (Baxter's Hollow) where the creek crosses the line between sections 32 and 33 (T.11N., R.6E.). The other was procured from the north branch of Honey Creek in the S. E. corner of section 11 (T.10N., R.4E.) of the same quadrangle.

Otter Creek heads in an area composed of about 90 per cent quartzite rock, the remainder being mainly sandstone and dolomite. Honey Creek, on the other hand, runs over moraines composed principally of sandstone (95 per cent) cemented with dolomite, with lesser amounts of quartzite (Wanenmacher, Twenhofel, and Raasch, 1934).

Water from both creeks was collected in five-gallon jugs. That which was used for the first series of tests was packaged in polyethelene bags, which were then sealed with an electric curling iron. Waxed cardboard containers were substituted for the polyethelene bags in later tests. The filled containers were placed in a deep freezer and kept until used. Before a test was run, the ice was melted and allowed to come to room temperature.

It was the immediate intent of this experiment to determine if it were possible to obtain an olfactory discrimination between Otter Creek and Honey Creek by the bluntnose minnow. The minnows were trained in such a manner that they learned to associate the odor of one of the streams with food and the odor of the other with punishment.

The equipment used by the authors (1949 and 1950) was suitable for conducting this series of training tests. It consisted of several seven gallon aquaria, each with a siphon-airlift circulation system installed in both ends (fig. 1). Water was siphoned from the aquarium, returned by air pressure, and discharged into a 6-inch funnel which was suspended above the tank. The funnel was connected to a glass tube which lay across the end of the aquarium. Perforations in the tube directed the incoming water across the bottom of the aquarium. Water from the jet on one side flowed only about halfway across, because there it met the stream from the other end, and both were deflected upward. This produced two currents or convection cells, each of which involved one half of the tank. Water samples containing the odors were introduced into the aquarium by means of a separatory funnel, which was connected to the siphon tube after it left the tank.

An objection to the two-electrode punishment system, as described by the authors (*l.c.*), was overcome by introducing a third electrode. Formerly, it was difficult to punish a fish which entered the end zone more than two to four centimeters above the electrodes. Also, a fish between the electrodes was apt to be injured, as a shock was administered. With a third electrode, located about two inches above the one in the corner, it was possible to punish, without injuring, any fish entering the end zone below the level of the new electrode. Thus, a region (2″ x 2″ x 6″) bounded on the bottom by the two electrodes on the floor of the aquarium was designated as the "end zone," that is, the place where the fish were fed or punished by electric shock (2.3 volts; 20 milliamperes) depending on which odor was being introduced. Also, higher voltages could be used without adversely affecting the fish, thereby impressing the training to the negative odor.

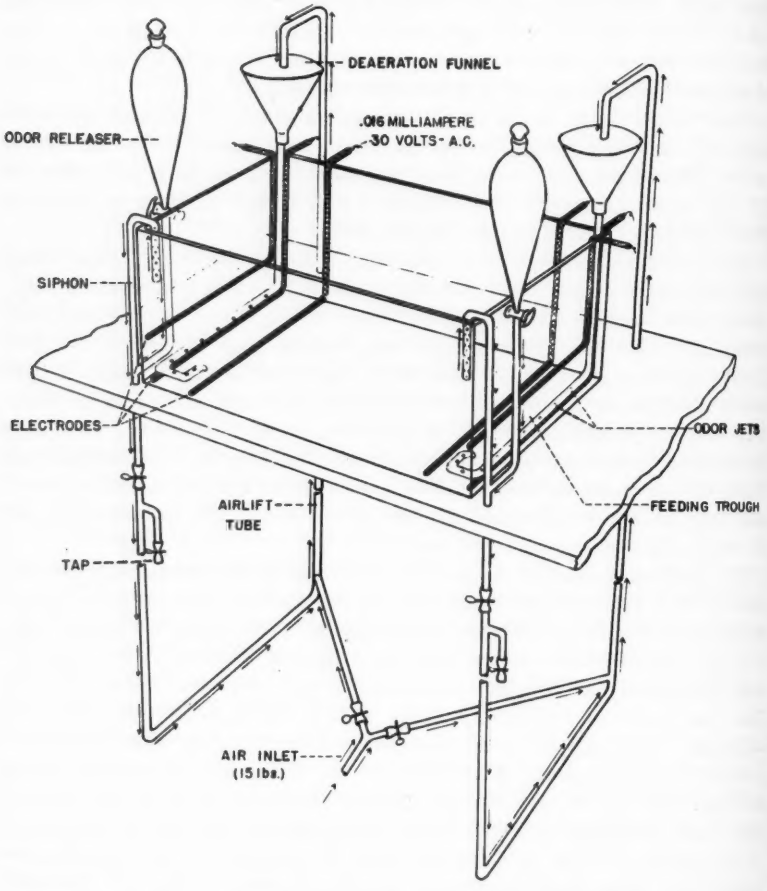

FIGURE 1. Experimental tank with siphon-airlift circulation system.

The fish were rewarded by introducing food, pressed on perforated celluloid strips, into the end zone. Since, in this method of training, hunger is the principal motivating force, tests were timed at those intervals when hunger motivation was at its greatest. An attempt was made to test at different times each day, and to feed no more than was necessary for the well-being of the experimental animals. The fish were fed very heavily every sixth day, and tests omitted on the seventh, because a mere subsistence diet such as this would, in time, have harmed them.

With a set of electrodes and an odor outlet in each end of the aquarium, it was possible to randomize the presentation of odors. A table of random numbers (Snedecor, 1948) was used to determine for each day which odor was to be presented first, and from which end of the aquarium. In order to

eliminate cueing to the operator, the fish were blinded by chemical cautery (injection of phemerol into the posterior chamber of the eye).

To eliminate any extraneous stimuli that the fish might receive during training, the following additional precautions were taken:

1. The aquaria were insulated from gross mechanical shocks by mounting them on sponge rubber.

2. Introduction of the odor was accomplished in such a manner that it did not alter the dimensions of the stream of water which was constantly flowing into the aquarium.

3. The observer whispered scores to a tape recorder over a telephone system to avoid any association with changes in pitch or rapidity of the observer's speech.

Fishes of two aquaria received positive training to water of Otter Creek, that is, fish were fed immediately after the test, and negative training to water of Honey Creek, that is, fish were punished by a light electrical current if they entered the end zone during the time this water was being introduced. Two other aquaria were trained to the same two odors, but with opposite meanings; Honey Creek was the positive odor; Otter Creek, the negative. If training were accomplished the fish should eventually associate food with the positive scent, and therefore enter the end zone. The negative scent would be associated with punishment and the fish should stay out of the end zone.

It should be pointed up that the fish were fatigued to the natural odors of the aquarium so that introduction of an unfamiliar water was immediately detected if in a perceptible concentration. In order to assure a forceful stimulus the creek water was diluted only by one-half when it was put into the separatory funnel. A minimal detectable concentration was not established.

The scoring procedure was essentially the same as that previously reported with but few modifications. A new method of recording the scores, using I.B.M. cards, was devised and will be described in a future paper.

Each fish was given a distinctive mark by attaching a bead to its back with a tantalum wire suture. Since each bead was of a unique color it was an easy matter to record the reaction of the individual fish.

The score for the entire aquarium was obtained from the mean scores of the six fish composing the group. Scores for three different measurements of reaction were obtained:

1. *Test:* Scores recorded during a sixty second test period coinciding with exposure to the odor. The test period was divided into intervals of 5 seconds. A fish scored once if it entered the end zone in a 5-second interval. Additional entries by the same fish in this same period were not counted. Thus, one fish could accumulate a total of twelve points per test. Arbitrarily these points were expressed as sixty seconds, that is, five seconds allotted to each point.

2. *Hesitation:* Scores for those five-second intervals intervening between the time the fish perceived the odor until it entered the end zone. Percep-

tion of the odor is manifested by a sudden change in behavior of the fish. It is especially obvious in trained fish and is easily recognized by the observer after short familiarity with the fish.

3. *Pretest:* Scores of random movement during a sixty second period prior to introduction of the odor.

REACTION TO TRAINING ODORS

Positive and negative tests were given daily and the fishes' scores recorded.

At the outset the fishes showed natural unlearned orientative responses for the odor of either creek, that is, they entered the end zone more often during the introduction of the odor on either the negative or the positive test than during the pretest. Not until later in the training sequence did they discriminate between them.

Tables 1a and 1b give records of activity according to the three items of behavior listed above. It is evident that the minnows learned equally well irrespective of whether Otter Creek (draining quartzite watershed) was positive (table 1b) or negative (table 1a). A noticeable degree of discrimination was accomplished in a month of training. Data presented in the tables are averages for training at the end of each month.

After reaching the discrimination level shown in tables 1a and 1b training was continued for two more months in order to attain the maximum level of discrimination, or the plateau in the learning curve.

RETENTION OF LEARNING

If salmon, also, are able to detect characteristic stream odors, and if these odors are to serve as orienting influences on migrating fishes, the early associations to the odor must be retained during the four-year sojourn at sea.

An attempt was therefore made to determine the length of retention of discrimination in the bluntnose minnow. Daily training was stopped and odors were presented weekly, without reward or punishment. After six weeks the fish were confusing the two odors so completely it was apparent that discrimination no longer existed (table 2a).

That this method of testing does not produce a clear-cut measure of actual retention is well known to psychologists. The procedure followed involves detraining through repeated testing, and through interference habits set up by routine daily feeding between tests. That is, an animal which has been trained to associate food with an odor, will be subjected to the reverse of this training process if fed without prior introduction of the odor. Thus, the minnows, during these tests, were actually being detrained, and the results of the tests can only be considered to be an absolute minimum indication of true retention.

It should be kept in mind that the life span of the bluntnose minnow is only two years. Hence, training was started at the senile stage, whereas salmon would be conditioned to the stream odor while young.

TABLE 1a

MEAN TRAINING SCORES IN SECONDS FOR FISHES REACTING TO ODOR STIMULI WHERE THE POSITIVE ODOR¶ WAS WATER FROM OTTER CREEK, AND THE NEGATIVE ODOR† WAS WATER FROM HONEY CREEK

Tank No.:		7			8		
Month of training*	Odor tested	Pretest‡	Test§	Hesitation‖	Pretest	Test	Hesitation
First	positive	63	97	69	39	142	43
	negative	66	46	104	35	61	82
Second	positive	85	181	55	57	160	47
	negative	73	20	200	52	18	196
Third	positive	68	194	46	43	176	31
	negative	47	21	217	40	19	209
Sixth	positive	47	200	6	39	242	9
	negative	39	15	241	40	13	226

TABLE 1b

POSITIVE ODOR: HONEY CREEK; NEGATIVE ODOR: OTTER CREEK

Tank No.:		2			3		
Month of training*	Odor tested	Pretest	Test	Hesitation	Pretest	Test	Hesitation
First	positive	25	76	76	40	113	34
	negative	58	52	132	68	43	146
Second	positive	47	123	56	87	156	35
	negative	42	40	180	76	44	137
Third	positive	43	146	47	64	182	23
	negative	57	27	203	39	19	129
Sixth	positive	46	343	6	34	336	0
	negative	31	13	285	40	17	273

*Records for tests made at this stage of training.

¶Positive odor: The odor with which fish had been trained to associate reward.

†Negative odor: The odor with which fish had been trained to associate punishment. No punishment accompanied these tests.

‡Pretest: Scores from random movement during 60 seconds preceding test.

§Test: Scores during 60 second period coinciding with exposure to odor.

‖Hesitation: Scores for 5 second intervals from time they perceive the odor until they enter the scoring zone.

Early conditioning in lower animals is known to influence their adult habits. Thorpe (1938) concluded that the ichneumon fly, Nemertis, has an inherited oviposition response to larvae of the flour moth, Ephestia, but not to larvae of the bee-wax moth, Meliphora. However it responds to the latter if raised on it during the larval feeding period, and given equal opportunity to oviposit on either species. And, a case closer to the point, Fish and Hanavan (1948) furnish good evidence that runs of salmon were established in renovated streams from migrants unable to pass the newly

TABLE 2a

EXTINCTION TESTS: SCORES IN SECONDS FOR OLD FISHES REACTING TO ODOR STIMULI OF THE TRAINING ODORS

Tank No.:		2			3			8		
Days after training*	Water tested	Pretest	Test	Hesitation	Pretest	Test	Hesitation	Pretest	Test	Hesitation
7	Honey Creek†	50	320	0	50	325	10	40	15	150
	Otter Creek	20	40	220	50	15	220	35	235	0
17	Honey Creek	60	190	0	45	325	10	65	70	180
	Otter Creek	60	50	125	70	60	115	80	270	0
29	Honey Creek	50	150	25	60	175	60	70	95	125
	Otter Creek	70	145	60	40	100	85	65	90	90
39	Honey Creek	110	115	80	45	75	75	115	125	130
	Otter Creek	120	130	120	25	75	130	120	140	115

*Records for tests made on this day after training ceased.

†In tanks 2 and 3, Honey Creek was the positive odor; Otter Creek, negative. In tank 8, Otter Creek was the positive odor; Honey Creek, negative.

built Grand Coulee dam. It appears here that eggs from these relocated fish yielded survivors which responded to some characteristic of an adopted stream to which they had been conditioned.

It was then felt that it would be desirable to know whether or not the retention of learning in young fishes is longer than in old, which should be the case if this hypothesis is to be applied to salmon. For this reason, an entirely new set of yearling minnows was trained to the level of discrimination previously attained with the same creek waters and methods described above. It was found that these fish were able to discriminate between the odors for fifteen weeks after cessation of training (table 2b).

TABLE 2b

EXTINCTION TESTS: SCORES IN SECONDS FOR YOUNG FISHES REACTING TO ODOR STIMULI OF THE TRAINING ODORS

Tank No.:		2		5	
Days after training	Water tested	Test	Hesitation	Test	Hesitation
0	Otter Creek*	322	8	22	297
	Honey Creek†	21	304	315	17
52	Otter Creek	276	15	102	254
	Honey Creek	53	272	304	24
66	Otter Creek	261	34	43	266
	Honey Creek	29	256	205	51
95	Otter Creek	93	97	102	119
	Honey Creek	71	134	182	96
105	Otter Creek	74	114	107	76
	Honey Creek	82	127	121	90

*Otter Creek was the positive odor in tank 2, and the negative odor in tank 5.
†Honey Creek was the positive odor in tank 5, and the negative odor in tank 2.

There is a record of true retention of learning in fishes, where detraining was not involved. Stetter (1929), in a study of sound discrimination, reported that the minnow *Phoxinus laevis* responded to tones 229 days after it had been conditioned by training.

SEASONAL INFLUENCE ON CHARACTER OF ODOR

It was postulated, in the introduction, that a stream must retain its characteristic odor throughout the year, as well as over a period of years, in order to be of value in explaining parent stream behavior. To test the possibility of seasonal changes in odor, samples were collected during winter and presented to fish that had been trained to water from the same streams which had been collected in summer. The fish responded equally well to this water, indicating that the odor characteristics recognized by the fish in these two streams did not lose their identity with the change in season.

EVIDENCE OF OLFACTORY DETECTION OF STREAM ODORS

To determine if these differences in water are perceived by tissues of the fish's nose, the olfactory capsules of trained fishes were destroyed by heat cautery. After the wound had healed, these fish were again tested with the training odors. There was no response; nor did they participate in the reaction when placed in an aquarium with normal, trained minnows. It can thus be seen that a reaction to the substance is dependent on the olfactory system and on individual perception of the odor, and is not a "follow the leader" phenomenon.

That the latter is true can also be shown by placing a blinded, but otherwise normal, fish, which has received positive training to one odor and negative training to the other, in an aquarium with fishes which have received the reverse training. When one of the training odors is then introduced into the aquarium, the odd fish exhibits a response which is the opposite of that being demonstrated by the resident fishes.

NATURE OF THE ODOR

With this proof that the olfactory receptors were stimulated by a property of the creek water, it was logical to wonder what the substance was. A standard chemical analysis was made of samples of the water which had been frozen (table 3). While chemical differences existed, it must be remembered that no fishes have ever been trained to differentiate, by smell, various levels of nitrogen, phosphorus, alkalinity, or pH. Of significance in these analyses is the zero CO_2, because Powers (1939) postulated that salmon might be able to follow gradients of CO_2 in streams or of water masses. If the salmon responds as does the minnow, this factor can be ruled out as a signal to a migratory route.

Differences in total organic nitrogen of the two streams were quite marked. Since most odorous compounds are organic in nature, it seems quite likely that the elements detected by the minnows may be located in this fraction.

INTERPRETATION OF GENERALIZATION TEST DATA

Fish trained to a positive and negative odor may be tested against a new third odor. This technique is known as a generalization test, commonly used in psychological experiments. Here it is presumed that the degree of similarity between a new stimulus and either of the stimuli to which the fish have been conditioned, is determined by observing how nearly the former evokes the behavior associated with either of the learned stimuli.

In these experiments, as mentioned previously, fishes of two aquaria received positive training to water of Honey Creek, and negative training to water of Otter Creek, while two other aquaria were trained to the same two odors but with opposite meanings attached. Thus a third (generalization) odor which produces a negative response in fishes of one aquarium, should

TABLE 3

CHEMICAL COMPOSITION OF THE TEST WATER, EXPRESSED IN P.P.M.

	Otter Creek	Honey Creek
NH_3-N	0.04	...
NO^3-N	0.23	0.59
NO^2-N	0.16	0.50
T.O.N.	0.23	0.59
Sol. P.	0.02	0.15
pH	8.2	8.5
Alkalinity $CaCO_3$	19.0	25.0
Free CO_2	0.0	0.0

produce a positive reaction in the fishes of the opposite aquarium. This, however, is not always the case (authors, *l.c.*). An odor which evokes a negative response in an aquarium in which Otter Creek is negative, may not produce a positive reaction in the aquarium in which Otter Creek is positive. On the other hand, a new odor which is accorded a positive reaction in one aquarium, will always receive negative treatment in the aquarium which has received the opposite training. This would imply that avoidance of electrical shock, which in training is associated with the negative odor, is a stronger motivating factor than hunger. A negative reaction to a generalization odor in both aquaria of an oppositely trained pair, may be the result of an avoidance response to a strange odor. A new (generalization) odor can only be considered similar to a given training odor when it produces the same reaction as that training odor in both aquaria of an oppositely trained pair.

RESPONSE TO ASH RESIDUE

The first generalization test was designed to determine whether the odor to which the fishes responded was organic or inorganic in nature (table 4)[2]. The ash residue of each stream was dissolved in distilled water, diluted to the original volume, placed in containers, frozen and tested with the trained minnows precisely as before. A complete set of experiments proved that the fish did not associate the residue with either of their training odors since their scores were within the range of the control tests on distilled water. This established more clearly that the odorous stimuli might be either in the organic fraction or an organic-inorganic complex.

RESPONSES TO DISTILLATES

(a) To fraction volatile at 100°C. and its residue.

Water from both creeks was distilled at 100°C. at atmospheric pressure and tests were conducted with both the distillate and the rediluted residue.

[2]With reference to tests on organic fractions, table 4 lists results only of two aquaria of a duplicate set. To save space, duplicates were not included since they did not add to the significance of the results.

TABLE 4

MEAN TRAINING SCORES IN SECONDS FOR FISHES REACTING TO ODORS OF OTTER CREEK AND HONEY CREEK AND TO VARIOUS FRACTIONS OF THESE WATERS.*

Tank No.:	1			4		
Odor tested	Pretest	Test	Hesitation	Pretest	Test	Hesitation
Otter Creek	45	326	5	40	22	291
Honey Creek	48	18	298	46	313	14
Inorganic fraction of Otter Creek	....	45	145	....	88	158
Inorganic fraction of Honey Creek	....	55	178	....	75	112
Distillate of Otter Creek (100°C)	....	56	176	....	49	164
Distillate of Honey Creek (100°C)	....	50	182	....	48	159
Residue of Otter Creek (100°C)	....	61	190	....	66	177
Residue of Honey Creek (100°C)	....	58	183	....	64	174
Distillate of Otter Creek (25°C)	....	276	36	....	27	251
Distillate of Honey Creek (25°C)	....	24	247	....	292	30
Residue of Otter Creek (25°C)	....	149	78	....	56	217
Residue of Honey Creek (25°C)	....	32	200	....	184	94
Residue and distillate of Otter Creek (25°C)	....	297	21	....	36	247
Residue and distillate of Honey Creek (25°C)	....	20	276	....	304	21
Distilled water control	....	40	155	....	75	160

*Otter Creek was the positive odor in aquarium No. 1, and the negative odor in aquarium No. 4.

Honey Creek was the negative odor in aquarium No. 1, and the positive odor in aquarium No. 4.

The fish proved to be as oblivious of these odors as of the above (table 4); the chemical stimulant apparently being destroyed by heat. Control tests with pyrex distilled water produced scores of the same order of magnitude indicating that any reaction produced was due to normal handling of the frozen water. It should perhaps be mentioned that in the generalization tests described so far, difficulty was experienced in trying to determine whether the scores were the result of random movement of the fishes or of responses to the solution introduced. The characteristic excitatory reaction, which was described as accompanying detection of one of the training odors, was completely lacking.

(b) To fraction volatile at 25°C. and its residue.

The next series of tests was conducted with the residue and distillate of water that had been vacuum distilled (pressure: approximately 22 mm. Hg.) at 25°C. (table 4).

The distillate and its residue were first mixed and tested to determine if the process of distillation had somehow altered the nature of the odor. It was noted by the observer that the intensity of the reaction to the positive odor was diminished. However, it was not possible to demonstrate a significant difference ($X^2 = 1.46$, $p = 0.49$) between these scores and those of the normal positive odor.

The fishes were then subjected to each of the two fractions separately. As can be seen from table 4, the scores of the reaction to the distillate more nearly resembled the scores of the response to the original water than did those of the redissolved residue. In fact, there was no significant difference between positive scores for the distillate and for the original water of either creek ($X^2 = 2.23$, $p = 0.4$), but a highly significant one between scores for the rediluted residue and the original water ($X^2 = 66.95$, $p = 0.0001$). These comparisons were made only on positive test scores since, as was mentioned previously, factors other than a similarity between odors may produce a negative response.

This biological assay, then, indicated that the odorous stimulant was contained in the volatile fraction and might thus be presumed to be an aromatic substance.

SIGNIFICANCE AND APPLICATION

Most of the evidence for the reliability of parent stream behavior in fishes is found in the literature on the salmon. When it was decided to initiate a series of experiments to attempt to discover the mechanism behind parent stream behavior, therefore, they were designed to lead eventually to a series of actual tests in the field with salmon.

In this research, one major barrier to the hypothesis that fish orient to their home streams, has been explored. That is, it was shown that some streams, at least, have odor characteristics which can serve to produce persisting differential responses in certain fishes. Furthermore, the results of generalization tests indicate strongly that the odors of streams are aromatic substances present in the volatile organic fraction. However, our evidence for olfactory discrimination of stream water by fishes does not constitute proof that parent stream behavior is not also controlled by other factors.

Scheer (1939) in his review of the homing instinct writes:

> The existence of a certain ability to follow a definite migratory course leads naturally to the question, what sense or senses are involved?... Ward (1921a, b, 1939a, b) studied *O. nerka* in the Copper River, Alaska, and concluded that when presented with a choice between two tributaries, the salmon invariably chooses the one with the cooler water.... Powers (1939) has attributed the direction taken by *O. nerka* to (a) gradients of salinity in the sea, and (b) gradients of CO_2 tension in the sea and in rivers. Although the suggestions made by these writers are of some value in indicating possibilities, neither has taken into consideration the fact that a run of fishes, whether in the sea or in a river, may divide, some passing into one river or tributary while others continue in their previous course. Neither author is willing to agree with the parent stream theory as stated above.

After citing Craigie's inconclusive results (see Introduction) Scheer continues:

> We must then say that at present we know little or nothing about any mechanism which might enable the salmon to find his way to a particular stream or tributary. Indeed we may say as Sumner (1939) has, that it is difficult to see how any known sense or combination of senses would be adequate, in view of the fact that variations in a certain property of a given stream might be greater from year to year than would be the differences between the stream and a nearby one. Studies directed at this feature of the "homing" question should prove of the greatest of interest.

In our studies we believe to have presented evidence which answers Sumner's objections. Also the mere fact that it can be demonstrated that fishes are attracted or repelled by substances such as CO_2 (Powers, 1943) does not signify that the salmon are responding to it in homing. Indeed, it would seem to preclude that possibility. If this were the case salmon might be expected to follow a gradient regardless of their origin.

One of the characteristics which a stream must have to fulfill the conditions of our thesis is that the substance, to which the fishes are responding on their return journey, must remain detectable even though the stream be changed severely in chemical and physical characteristics. Salmon continue to return regardless of pollution, floods, and changes in weather. These things do alter the materials in the stream, but on the basis of our evidence it appears that aromatics derived from the vegetation and soils of the watershed lend a distinctive odor which can be perceived, learned and recognized again after a protracted period of non-exposure. The aromatic characteristic of a watershed, filtering into the stream, might be surmised to remain constant over long periods.

Additional collateral indications of the importance of the sense of smell in the life of a fish comes from a large series of studies by von Frisch (1941) and his students. Their results attest the extreme sensitivity of the fish nose to natural substances, for they show that fish have been found capable of recognizing one another by scent, and that they may be alarmed at extremely dilute emanations (Schreckstoff) from injured fish skin. It would seem too that the acuity of the sense of smell in fishes is of similar sensitivity as that of dogs and insects where but a few molecules stimulate the end organ. In contrast, the common chemical sense (Powers, 1943) and the ability to discriminate temperature differences (Dijgraff, 1940) are crude senses when compared with the olfactory system.

Techniques have been developed whereby it is now possible to hatch and maintain salmon through the fingerling stage in aquaria at Madison. Preliminary results from a set of experiments currently in progress indicate that the olfactory system of salmon is very acute, and that they can discriminate between stream odors.

A set of field experiments must also be undertaken to furnish final proof of the hypothesis herein contained. Of a number of possible methods of solution, one promises to be of some practical value. The hypothesis

could be tested by exposing salmon to a constant, artificial odor through the fingerling stage and then determining if the fish conditioned in a hatchery could be decoyed to a neighboring stream upon return from the sea. Should this be the case, it would aid in salvaging the declining salmon runs where new dams may obstruct passage to their parent streams.

Before field experiments can be realized, however, it is necessary to learn a great deal more about the olfactory responses in fish, and about the other systems which may be involved in migratory behavior.

Other field experiments are being planned which will be aimed toward measuring the preciseness and determining the means by which salmon choose one tributary over another.

SUMMARY

1. Various theories have been advanced to explain the mechanism by which migrating salmon return to their parent stream. One of these postulates the presence of some characteristic odor of the stream which guides the returning migrants. This theory presents two distinct problems:

(1) Do streams have characteristic odors to which fish can react? If so, is the odor organic or inorganic in nature, or a combination of both?

(2) Can salmon detect and discriminate between such odors, if they do exist?

2. In an attempt to answer the first question, a conditioned response training program was started with the bluntnose minnow. The fishes were able to discriminate successfully between chemical differences of two Wisconsin creeks after two months' training.

3. Extinction tests indicated that these minnows would respond to the stream odors after a "forgetting period," which was longer in fishes trained when young than in those trained in senility.

4. Heat cautery of the olfactory epithelium produced fish which were no longer able to respond to the training odors; proving that olfaction was the sole means of discrimination in these tests.

5. Chemical analysis of the stream waters indicated a total absence of CO_2; proving that this compound was not that which was detected.

6. Fractionation of the stream waters proved that the fish did not react to the inorganic ash, or to the distillate or residue of water fractionated at 100°C. They reacted to the distillate but not the residue, of water fractionated by vacuum distillation at 25°C.; a strong indication that the odorous stimulant is a volatile, aromatic substance.

7. Preliminary tests with salmon proved that they can detect the stream odors, and that they were able to discriminate between them.

8. It is postulated that the nature of the guiding odor must be such that it have meaning only for those salmon conditioned to it during their fresh-water sojourn. Any substance which was merely a general attractant could not guide salmon to their "home" tributary.

LITERATURE CITED

Clemens, W. A., R. E. Foerster and A. L. Pritchard, 1939, The migration of Pacific salmon in British Columbia waters. From: The Migration and Conservation of Salmon. Publ. Amer. Ass. Adv. Sci. 8: 51–59.

Craigie, E. Horne, 1926, A preliminary experiment upon the relation of the olfactory sense to the migration of the sockeye salmon. (*Oncorhynchus nerka*, Walbaum). Trans. Roy. Soc. Canada. 20: 215–224.

Dijgraff, Sven, 1940, Untersuchungen über den Temperatursinn der Fische. Zeits. für vergl. Physiol. 25 (5): 587–605.

Fish, Frederic F. and Mitchell G. Hanavan, 1948, A report upon the Grand Coulee fish-maintenance project 1939–1947. U. S. Fish and Wildlife Service, Special Scientific Report No. 55, 63 pp.

Frisch, K. v., 1941, Die Bedeutung des Geruchsinnes im Leben der Fische. Naturwiss. 29(22/23): 321–333.

Hasler, Arthur D. and Warren J. Wisby, 1950, Use of fish for the olfactory assay of pollutants (phenols) in water. Trans. Amer. Fisheries Soc. 79: 64–70.

Kyle, Harry M., 1926, The Biology of Fishes. Sidgwick and Jackson, Ltd., London. 396 pp.

Powers, Edwin B., 1939, Chemical factors affecting the migratory movements of the Pacific salmon. From: The Migration and Conservation of Salmon. Publ. Amer. Ass. Adv. Sci. 8: 72–85.

Powers, E. B. and R. T. Clark, 1943, Further evidence on chemical factors affecting the migratory movements of fishes, especially the salmon. Ecology 24(1): 109–113.

Scheer, Bradley T., 1939, Homing instinct in salmon. Quart. Rev. Biol. 14(4): 408–430.

Scheuring, Ludwig, 1930, Die Wanderungen der Fische. Zweiter Teil. Ergebnisse der Biologie 6: 4–304.

Snedecor, George W., 1946, Statistical Methods. Iowa State College Press. Ames, Iowa. 485 pp.

Stetter, H., 1929, Untersuchungen über den Gehörsinn der Fische, besonders von *Phoxinus laevis* L. und *Amiurus nebulosus* Raf. Zeits. vergl. Physiol. 9: 339–477.

Sumner, F. B., 1939, Human psychology and some things that fishes do. Scientific Monthly 49: 245–255.

Thorpe, W. H. and F. G. W. Jones, 1938, Olfactory conditioning in a parasitic insect and its relation to the problem of host selection. Proc. Roy. Soc. London. Biological Sciences. Series B 124: 56–81.

Walker, Theodore J. and Arthur D. Hasler, 1949, Detection and discrimination of odors of aquatic plants by *Hyborhynchus notatus* (Raf.). Physiol. Zool. 22: 45–63.

Wanenmacher, J. M., W. H. Twenhofal, and G. O. Raasch, 1934, The paleozoic strata of the Baraboo area, Wis. Amer. Jour. Sci. 28(163): 1–30.

Ward, Henry B., 1921a, Some of the factors controlling the migration and spawning of the Alaska red salmon. Ecology 2: 235–254.

1921b, Some features in the migration of the sockeye salmon and their practical significance. Trans. Amer. Fisheries Soc. 50: 387–426.

1939a, Psychology of salmon. Proceedings of the Washington Academy of Sciences 29: 1–14.

1939b, Factors controlling salmon migration. Migration and Conservation of Salmon. Publ. Amer. Ass. Adv. Sci. 8: 60–71.

White, H. C., 1934, Some facts and theories concerning the Atlantic salmon. Trans. Amer. Fisheries Soc. 64: 360–362.

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STRUCTURAL HETEROZYGOSITY IN A VERY RARE SPECIES OF GRASSHOPPER[1]

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INTRODUCTION

Species of animals which are excessively rare or localized are interesting since they may provide critical evidence in connection with a number of problems in fundamental evolutionary theory. Such species are, however, of several different categories. On the one hand we have those which are restricted to some very small territory (*e.g.* an oceanic island) surrounded by a region which the species could not conceivably inhabit. The smallness of the distribution area in this case is not due to any inability of the species to adapt itself. On the other hand there are certain species which inhabit relatively minute areas of the earth's surface, to which they are apparently confined by their inability to adapt themselves to the ecological conditions of the surrounding terrain. Species of this type may be confined to a single such area or to several small areas between which the species does not occur.

Most very rare species show little obvious variation, or at any rate their populations are not visibly polymorphic, and it has been generally assumed, on theoretical grounds, that where the breeding population is of the order of only a few hundred individuals or less there will be a strong tendency towards genetic homozygosity. The present note deals with a case in which an extremely rare species shows a very high degree of structural heterozygosity in its chromosomes; we do not believe that any comparable case has been previously recorded.

Among the North American grasshoppers there are a number of species which have been collected at a single locality, or very few localities, and which are represented in collections by single individuals, or by very small series. Certain of these (such as *Spharagemon superbum* known only from two individuals collected at Katherine, Willacy Co., Texas) are forms which occur not far from the Mexican border, and may have an extensive distribution south of the border, where little collecting has been done. *Shotwellia isleta* is only known from two individuals, one from near Albuquerque, N. M., the other from Gomez Palacio, Durango, Mexico, approximately 700 miles to the south. It is at least possible that this species may be locally common in certain areas of Mexico that have not been visited by collectors.

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Such considerations do not apply in the case of species which occur in thoroughly collected areas of the United States, remote from the Mexican border. It is fairly certain that *Anconia caeruleipennis,* known only from the vicinity of Walker Lake, Nevada, is a species of extremely restricted distribution, and is probably represented by very few individuals at the present time. The Decticine katydid, *Zacycloptera atripennis,* also known only from Walker Lake, is in the same category. These species are almost certainly "relics" which had a wider distribution in Pluvial times, when the Great Basin area contained extensive lakes, represented at the present time only by a few widely scattered bodies of water, of which Walker Lake is one of the largest.

The present note deals with another very rare species of grasshopper, *Pedioscirtetes nevadensis* (subfamily Truxalinae). The species was described by Thomas (1873) from material collected during the 1871 Geographical Survey West of the 100th Meridian. The exact locality where the material was obtained is not known, but may have been in southeastern Nevada, although the species has never been subsequently recorded from that state. Bruner (1890) described *"Pedioscertetes" pulchella,* a synonym of *P. nevadensis,* from specimens collected on lava flows at Birch Creek, Idaho, apparently feeding on *Grayia polygaloides* (Chenopodiaceae). Ball *et al.* have recorded the species from near Springerville and near Flagstaff, Arizona. These are the only published records of *P. nevadensis,* but in addition Rehn and Hebard collected it on the Paunsaugunt Plateau, west of Bryce Canyon, Utah. None of these orthopterists obtained the species in large numbers and at each locality where it has been found the population is apparently very small.

The colony of *P. nevadensis* on which the present work was based was discovered by the senior author in a *Pinus ponderosa* forest on volcanic soil, approximately 8 miles northeast of Flagstaff. This may be the same locality at which the late E. D. Ball collected the species. Adults and nymphs were present July 24-25, 1949, but only adults were collected August 15, 1950. The population was restricted to a small clearing, most of the insects being found on the Boraginaceous plant *Lappula coronata* Greene, which seemed to constitute the main diet. Ball *et al.* (1942) have stated that *P. nevadensis* is closely associated with the composite *Actinia richardsoni,* but this plant was not present at the locality where our collection was made.

The known distribution of *P. nevadensis* thus embraces a large area of northern Arizona, Utah, Idaho and (possibly) southeastern Nevada, but within this area the species has been taken at only a very few widely scattered localities, in spite of much intensive collecting by experienced entomologists such as Rehn, Hebard, Tinkham, Ball and others. Several orthopterists have looked for the species in the Flagstaff area without success. We are therefore justified in regarding *P. nevadensis* as a relic species which survives precariously in a few suitable localities. The colony collected by the senior author probably does not consist of more than a few hundred individuals in any one year (twenty-five were taken in 1949, twelve

in 1950, care being taken not to exterminate the colony). Under such circumstances a significant degree of inbreeding probably occurs, particularly since the species is somewhat sluggish in its movements. It was hence a complete surprise to find that *P. nevadensis* (or at any rate, this particular colony of it) exhibits a high degree of structural heterozygosity in certain of its chromosomes.

P. nevadensis is a rather isolated species taxonomically, the only other member of the genus, *P. maculipennis,* being not very closely related. *P. maculipennis* occurs on low, eroded hillsides in west Texas, southern New Mexico, southeastern Arizona and semi-desert areas of northeastern Mexico. Although a local species, the individual colonies usually show a high population-density, the species being frequently abundant in suitable environments.

The genus *Pedioscirtetes* is probably fairly closely related to *Acrolophitus,* and it is possible that a closer phyletic relationship exists between *Acrolophitus hirtipes* and *P. nevadensis* than between the latter and *P. maculipennis.*

The individuals in the Flagstaff population exhibit a certain amount of phenotypic polymorphism, but it is not known whether this has any genetic basis. The distinctness of the oblique cream-colored bands on the outer face of the hind femora is variable, and the dorsum of the pronotum, which is normally green, is tinged with purplish brown in some females. Bruner's types of *P. pulchella* had the tegmina mottled while in all our specimens they are uniformly green, but a similar variation occurs in *Acrolophitus hirtipes,* in which individuals from different localities may have the tegmina mottled or immaculate.

CYTOLOGICAL OBSERVATIONS

The cytology of *P. nevadensis* can best be understood after comparison with that of *P. maculipennis.* The latter species possesses a chromosome set which is of a type very characteristic of the sub-family Truxalinae, consisting of twenty-three acrocentric chromosomes in the male, that is, an X and eleven pairs of autosomes. The three smallest pairs of autosomes are considerably shorter than any of the others; since they are approximately the same size these three pairs cannot be distinguished from one another (fig. 1). The "short arms" of several of the autosomal chromosomes are quite conspicuous in various stages of mitosis and meiosis. Apart from the X, which is negatively heteropycnotic in the early spermatogonial divisions and again at the first meiotic metaphase, the chromosomes of *P. maculipennis* do not seem to contain extensive heterochromatic regions. Observations on resting stage nuclei and prophase stages of meiosis show that there is little heterochromatin in the autosomes. No structural heterozygosity was seen in nine individuals from Bottomless Lakes State Park, Chaves Co., New Mexico, the size of the three pairs of small chromosomes being apparently constant.

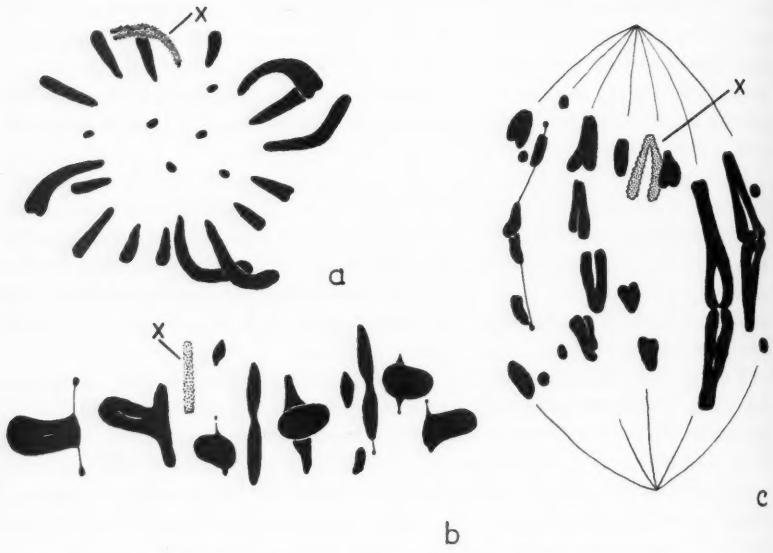

FIGURE 1. *Pedioscirtetes maculipennis: a,* spermatogonial metaphase; *b,* first metaphase; *c,* first anaphase.

P. nevadensis likewise has twenty-three chromosomes in the male, but the appearance of the three pairs of small chromosomes is quite different and varies from one individual to another. Each of these chromosomes appears to have acquired additional heterochromatin which is not present in *maculipennis.* The six small autosomes of *nevadensis* when seen in mitotic metaphase (fig. 2) are mostly V's or J's, considerably larger than the corresponding elements of *maculipennis,* but a variable number of them are small dots. At least two of these three autosomal elements may be represented either by a V or a dot. Thus the majority of individuals studied

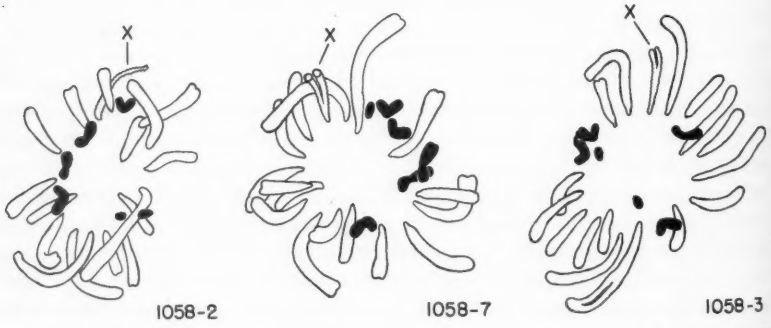

FIGURE 2. *Pedioscirtetes nevadensis:* spermatogonial metaphases of three different individuals. The six small autosomes are shown in black, the other autosomes in outline only.

had one or two of the three small pairs composed of elements of different sizes. Apparently, in such cases the smaller chromosome lacks a heterochromatic segment which is present in the larger one. Of nine individuals studied, six (nos. 1058-1, 1058-4, 1058-5, 1058-6, 1058-7 and 1058-9) had one of the three small pairs unequal (SL), the two other pairs being homozygous for the larger type of element (LL). We designate such individuals by the formula LL-LL-SL; it is not certain whether the SL bivalent is the same one in all six individuals. One grasshopper (1058-2) was a double heterozygote, *i.e.*, it had two unequal bivalents (formula: LL-SL-SL) and

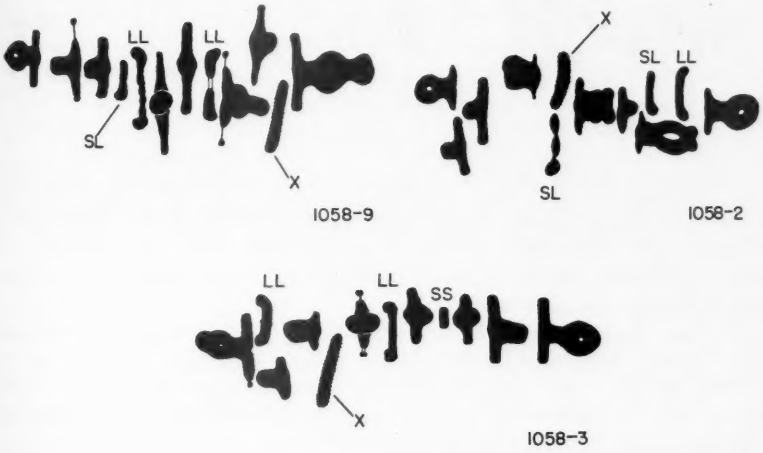

FIGURE 3. *Pedioscirtetes nevadensis:* first metaphases of three different individuals.

two (1058-3 and 1058-8) were structural homozygotes of the formula LL-LL-SS. The appearance of spermatogonial and meiotic divisions in these various types of individuals is shown in figs. 2–4. The "S" chromosomes are approximately one-third the size of the "L" chromosomes and seem to be effectively acrocentric. Thus the heterozygous bivalents are characteristically L-shaped and can be recognized before anaphase-separation has started.

Apparently the three small bivalents never form more than a single chiasma, regardless of whether they are structurally homozygous or heterozygous. Segregation of the S and L chromatids in unequal bivalents always takes place at the first meiotic division. This indicates that the cytological inequality is either situated proximal to the region in which the chiasma is formed or in the other arm of the chromosome.

That the small metacentric chromosomes of *P. nevadensis* do contain fairly extensive heterochromatic segments is clear from a study of resting primordial spermatogonia and early meiotic prophases, in which these heterochromatic segments can be clearly distinguished at certain stages. We believe that the "S" chromosomes lack certain heterochromatic seg-

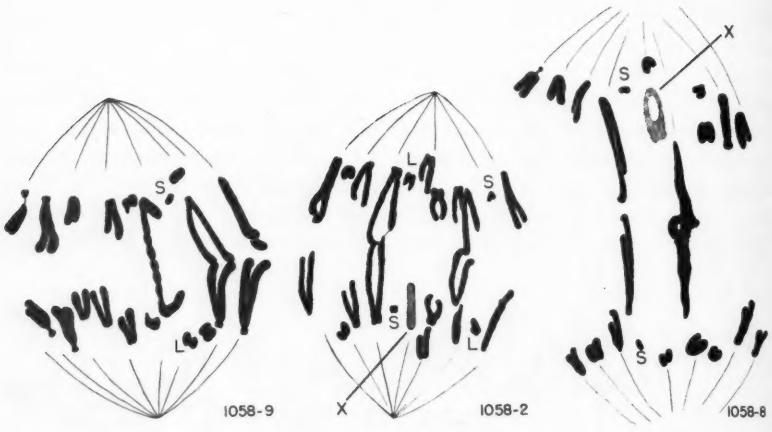

FIGURE 4. *Pedioscirtetes nevadensis:* first anaphases of three different individuals. In no. 1058-9 not all the chromosomes are drawn. In the case of the three small pairs of chromosomes the longer type is labelled L, the shorter type S.

ments present in the "L" chromosomes, but a detailed analysis is not possible with the available material.

We have made an attempt to compare the chiasma-frequencies of the two species of *Pedioscirtetes.* The results of this comparison are given in table 1. The difference in chiasma frequency between the two species is entirely due to the eight large bivalents, since the three small ones in both species invariably form a single chiasma.

DISCUSSION

Bivalents composed of two chromosomes of different sizes have been reported in a number of species of grasshoppers by Carothers (1913, 1931), Robertson (1915), Wenrich (1916), McClung (1928), Belar (1929), Hearne and Huskins (1935), Darlington (1936) and White (1951). Most of the species in which they have been recorded are widespread forms with large populations in which "drift" would not be an effective force. Moreover, in most of the cases previously studied, only a relatively small proportion of the population is heterozygous (*e.g.*, six out of a sample of fifty-six individuals of *Trimerotropis bilobata).* The present case is remarkable because of the very high

TABLE 1

CHIASMA FREQUENCIES OF THE TWO SPECIES

Number of chiasmata per nucleus	17	18	19	20	21	22	23	24	Mean
	Number of cells found								
P nevadensis	...	...	...	5	14	15	14	2	21.88 ±0.15
P. maculipennis	13	18	13	5	1	...	...	...	18.26 ±0.15

degree of heterozygosity present in a very small population. Although we did not attempt to determine the actual number of individuals present in the colony, we feel sure that it is so small that "drift" would inevitably have led to homozygosity unless rather strong selective forces were operating in favor of the heterozygotes. This implies, of course, that the various classes of homozygotes (or some of them) must be at a selective disadvantage. We did not find any LL-LL-LL individuals in the present sample. If the six single heterozygotes all belong to the same class (*i.e.*, if the unequal bivalent is the same one in all of them), then the absence of LL-LL-LL individuals from the sample is probably significant, and may indicate that they are of low viability. The extreme rarity of *P. nevadensis* is possibly due in part to the fact that it is a specialized feeder and in part to low fecundity (Ball *et al.* state that only six-eight eggs are laid in each "pod," compared with eleven-sixteen in *P. maculipennis*); but it may also result, to some extent, from a genetic mechanism involving lethality or near-lethality of certain categories of structural homozygotes.

Dobzhansky (1947, 1949, 1950—see also Dobzhansky, Burla and da Cunha, 1950) has put forward the view that adaptive polymorphism based on chromosomal rearrangements is especially characteristic of species having large effective breeding populations which are able to occupy a considerable variety of different ecological niches. Such a relationship may hold for the willistoni group of Drosophila and perhaps for some other groups of that genus. But in the present instance we have a species which is so rare that if it were a member of the genus Drosophila it would probably not have been discovered up to the present time exhibiting a high degree of chromosomal polymorphism. Of course, we have no proof that this polymorphism (which in any case is of quite a different type from the inversions of Drosophila species) is adaptive, but it seems almost inconceivable that it would have been preserved if it were not adaptive, in view of the population structure of the species. If we are able to collect *P. nevadensis* at other localities in the near future it will be most interesting to determine whether these other populations show the same type of structural heterozygosity as the one near Flagstaff.

SUMMARY

1. The very rare grasshopper *Pedioscirtetes nevadensis* has acquired certain heterochromatic segments in the three smallest pairs of autosomes which are not present in the corresponding autosomes of *Pedioscirtetes maculipennis*. These chromosomes, which are minute rods in the latter species, have been converted into much larger metacentric elements in the former species.

2. In *P. nevadensis* a large proportion of the individuals have one of these three pairs of chromosomes heterozygous for size, *i.e.*, one member of the pair carries a chromosome segment which is lacking in the other member. In some individuals a second pair of chromosomes is also heterozygous. We assume that this heterozygosity indicates the existence of a mechanism of heterosis of some kind.

3. The populations of *P. nevadensis* are of very small size, the species being one of the rarest North American grasshoppers. It is believed that this is the first account of cytological polymorphism in a species with such small populations.

4. The chiasma frequency of *P. nevadensis* is significantly higher than that of *P. maculipennis*.

LITERATURE CITED

Ball, E. D., E. R. Tinkham, R. Flock and C. T. Vorhies, 1942, The grasshoppers and other orthoptera of Arizona. Univ. Arizona Tech. Bull. No. 93: 255-373.

Belar, K., 1929, Beiträge zur Kausalanalyse der Mitose. II. Untersuchungen an den Spermatocyten von *Chorthippus (Stenobothrus) lineatus* Panz. Roux' Arch. Entw. Mech. Organ. 118: 359-484.

Bruner, L., 1890, New North American Acrididae, found north of the Mexican boundary. Proc. U. S. Nat. Mus. 12: 47-82.

Carothers, E. E., 1913, The mendelian ratio in relation to certain orthopteran chromosomes. J. Morph. 24: 487-511.

1931, The maturation divisions and segregation of heteromorphic homologous chromosomes in Acrididae (Orthoptera). Biol. Bull. 61: 324-349.

Darlington, C. D., 1936, Crossing over and its mechanical relationships in Chorthippus and Stauroderus. J. Genet. 33: 465-500.

Dobzhansky, Th., 1947, Adaptive changes induced by natural selection in wild populations of Drosophila. Evolution 1: 1-16.

1949, Observations and experiments on natural selection in Drosophila. Proc. VIII. Int. Genet. Congress, Hereditas suppl. vol., pp. 210-224.

1950, Mendelian populations and their evolution. Amer. Nat. 84: 401-418.

Dobzhansky, Th., H. Burla and A. B. da Cunha, 1950, A comparative study of chromosomal polymorphism in sibling species of the willistoni group of Drosophila. Amer. Nat. 84: 229-246.

Hearne, E. M., and C. L. Huskins, 1935, Chromosome pairing in *Melanoplus femur-rubrum*. Cytologia, 6, 123-147.

McClung, C. E., 1928, Differential chromosomes of *Mecostethus gracilis*. Zeits. Zellforsch. 7: 756-778.

Robertson, W. R. B., 1915, Chromosome studies. III. Inequalities and deficiencies in homologous chromosomes: their bearing upon synapsis and the loss of unit characters. J. Morph. 26: 109-141.

Thomas, C., 1873, Descriptions of new species of orthoptera collected in Nevada, Utah and Arizona by the expedition under Lt. Geo. M. Wheeler. Proc. Acad. Nat. Sci. Phila. 25: 295-297.

Wenrich, D. H., 1916, The spermatogenesis of *Phrynotettix magnus*, with special reference to synapsis and the individuality of the chromosomes. Bull. Mus. Comp. Zool. Harvard 60: 57-135.

White, M. J. D., 1951, Cytogenetics of orthopteroid insects. Advances in Genetics, 4 (in press).

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DEVELOPMENT OF HETEROSIS THROUGH NATURAL SELECTION IN EXPERIMENTAL POPULATIONS OF DROSOPHILA PSEUDOOBSCURA

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Most natural populations of *Drosophila pseudoobscura* are variable with respect to the gene arrangement in their third chromosomes. Several gene arrangements, differing in inversion of blocks of genes, coexist in many populations. Since the carriers of the different chromosomes interbreed freely, inversion heterozygotes and homozygotes are formed. The heterozygotes, provided that the two chromosomes with different gene arrangements are derived from the population of the same locality, are, as a rule, superior in adaptive value to the homozygotes (Dobzhansky, 1947a and b). This is true both in experimental and in natural populations (Wright and Dobzhansky, 1946; Dobzhansky and Levene, 1948). The adaptive superiority, heterosis, is however absent in some experimentally produced inversion heterozygotes which carry chromosomes derived from populations of geographically remote localities (Dobzhansky, 1949, 1950). The heterosis is, thus, not an intrinsic property of a gene arrangement. It is a result of interaction of complexes of polygenes carried in chromosomes with different gene arrangements. Within the population of each geographic region, the polygene complexes in the chromosomes have become mutually adjusted, or "coadapted," by a process of natural selection. The coadaptation results in a high fitness of the Mendelian population in which the chromosomes with the coadapted gene complexes occur.

The present article reports the results of the experiments which show that heterosis may indeed arise owing to the action of natural selection.

MATERIAL AND TECHNIQUE

The material used in this experiment consisted of twelve strains homozygous for the Chiricahua (CH), fifteen strains homozygous for Standard (ST), and twelve strains homozygous for the Arrowhead (AR) gene arrangements in the third chromosome. The CH strains were derived from wild ancestors collected in Chihuahua, Mexico, by Professor H. T. Spieth. The ST and AR strains came from the population of Piñon Flats, Mount San

[1] Experimental data by Th. Dobzhansky, mathematical analysis by H. Levene.
[2] Research of H. Levene under contract with Office of Naval Research.

Jacinto, California. These are the same strains which were used in the experiments of Dobzhansky (1950), and were carried as laboratory stocks during the interim. The construction of the population cages, and the techniques of sampling the experimental populations have been described by Wright and Dobzhansky (1946) and Dobzhansky (1947b, 1950).

VIABILITY AND ADAPTIVE VALUE

We define heterosis as adaptive superiority of heterozygotes over homozygotes. In our experiments, the adaptive superiority of inversion heterozygotes over homozygotes is detected and measured by observing the rates of change in the frequencies of chromosomes with different gene arrangements in experimental populations. A population with known proportions of two gene arrangements is allowed to breed in a population cage; samples of the eggs deposited by the flies in the cage are taken at desired intervals, and the chromosomes are examined in the larvae which develop from these eggs. The relative frequencies of the gene arrangements undergo, in many experiments, rapid changes from generation to generation. The occurrence of such changes proves that the different chromosomal types are not equal in adaptive value. Now, if the carriers of one gene arrangement were adaptively superior to those with another gene arrangement, one would expect that the less well adapted arrangement should eventually be lost, and the population should become uniform for the better adapted chromosomal type. This, however, is not what is actually observed in most experiments. Instead of one gene arrangement crowding out the other, an equilibrium is eventually reached in the experimental populations, at which both gene arrangements continue to occur with certain frequencies. The attainment of equilibria is expected if the highest adaptive values occur in the inversion heterozygotes, while the homozygotes are inferior in adaptive value (Wright and Dobzhansky, 1946).

The relative viabilities of the chromosomal types are not necessarily proportional to their adaptive values, although the viability is evidently one of the important variables which determine the adaptive value of a genotype. In non-lethal genotypes, a deficient viability may be compensated for by other qualities, such as a greater fecundity or a greater sexual activity (Dobzhansky, 1950; Wallace, 1948; da Cunha, 1949). The viability of a chromosomal type between the egg and the adult stage is measured, in our experiments, by observing the differential survival (or differential mortality) of known chromosomal types under competition (Dobzhansky, 1947, 1950). Between two and three thousand flies heterozygous for two gene arrangements, ST and CH, are introduced in a population cage. According to the first law of Mendel, 50 per cent of the eggs deposited in such a population must be inversion heterozygotes, ST/CH, 25 per cent homozygotes ST/ST, and 25 per cent homozygotes CH/CH. Because of the very large number of eggs deposited in the population cages, the competition for food is very stringent among larvae in the population cages; less than 10 per cent, and perhaps less than 1 per cent, of the eggs deposited in the

population cages produce adult flies. Now, if the mortality of the different genotypes is selective, the heterozygotes and the homozygotes may be more or less common among the adult flies which hatch in the population cages than they were among the eggs deposited by the parents. The relative viabilities of the genotypes are arrived at by comparison of the frequencies of the chromosomal types among the eggs deposited and among the adult flies which hatch in the population cages.

VIABILITY OF THE HYBRIDS BETWEEN STRAINS FROM CALIFORNIA AND FROM MEXICO

The Mexican Chiricahua strains (CH^M/CH^M) were outcrossed, in ordinary culture bottles, to Standard strains from California (ST^C/ST^C), or to California Arrowhead strains (AR^C/AR^C). On January 24, 1948, approximately 2300 ST^C/CH^M hybrid flies were placed in population cage 55, and a like number of AR^C/CH^M hybrids in cage 56. Both cages were kept at 25°C. The adult flies hatching in the cages in the next generation were isolated, and outcrossed in individual cultures to known CH homozygotes. The gene arrangement in the third chromosome was determined in six larvae in the progeny of each culture. This permits us to infer the chromosomal constitution of the parents which hatched in the cages. The results are summarized in table 1 (see also Dobzhansky, 1950).

Far from exhibiting a superior viability, the AR^C/CH^M heterozygotes are quite significantly inferior to the AR^C/AR^C homozygotes, and equal to, or even inferior, to the poorly viable CH^M/CH^M homozygotes. The performance of the ST^C/CH^M heterozygotes is different in the two sexes. Among the males, the homozygotes and heterozygotes do not differ significantly in viability. Among the females, the heterozygotes are decidedly inferior to the ST^C/ST^C homozygotes, and about equal to the CH^M/CH^M ones.

THE NATURAL SELECTION EXPERIMENT

If the adaptive values of the chromosomal types were proportional to the relative viabilities of their carriers, one could predict that the Mexican CH^M chromosomes would, given enough time, be eliminated by natural

TABLE 1

THE RELATIVE VIABILITY OF HOMOZYGOTES AND HETEROZYGOTES FOR CALIFORNIA AND MEXICAN CHROMOSOMES IN THE F_2 GENERATION OF THE INTERRACIAL CROSS

	Cage 55			Cage 56		
	CH^M/CH^M	ST^C/CH^M	ST^C/ST^C	CH^M/CH^M	AR^C/CH^M	AR^C/AR^C
Observed ♀♀	25	48	47	17	29	29
Viability	0.98 ± .25	1	1.87 ± .39	1.10 ± .35	1	1.91 ± .51
χ^2	0.01		9.10	0.10		5.96
Observed ♂♂	32	74	34	47	75	55
Viability	0.81 ± .18	1	0.86 ± .18	1.18 ± .23	1	1.39 ± .25
χ^2	0.97		0.50	0.77		3.29

selection in competition with the California ST^c and AR^c chromosomes. The experiment designed to test this prediction was started in the autumn of 1949. As before, the CH^M/CH^M strains were outcrossed in ordinary culture bottles to ST^c/ST^c or to AR^c/AR^c strains. On November 15th, some 2000 ST^c/CH^M hybrids were placed in cage 66 and AR^c/CH^M hybrids in cage 67. The cages were kept in an incubator at 25°C. From time to time samples of 300 chromosomes (150 larvae) were taken in each cage, as described before (Wright and Dobzhansky, 1946; Dobzhansky, 1950). The results are summarized in table 2 (see also figures 1 and 2 below).

TABLE 2

PERCENTAGE FREQUENCIES OF STANDARD (ST) AND ARROWHEAD (AR) CHROMOSOMES OF CALIFORNIA ORIGIN, AND OF CHIRICAHUA (CH) CHROMOSOMES OF MEXICAN ORIGIN IN THE POPULATION CAGES 66 AND 67.

	Cage 66		Cage 67	
	ST^c	CH^M	AR^c	CH^M
November 15, '49	50.0	50.0	50.0	50.0
M December '49	57.0	43.0	58.3	41.7
M January '50	63.7	36.3	61.7	38.3
L February '50	72.7	27.3	68.0	32.0
E April '50	76.7	23.3	66.3	33.7
M May '50	71.0	29.0	73.0	27.0
L June '50	72.0	28.0	76.6	23.3
L September '50	69.3	30.7	75.3	24.7
M November '50	64.3	35.7	76.0	24.0
M January '51	69.0	31.0	...	...

*E = early; M = middle; L = late.

In both experiments, the frequencies of CH^M chromosomes rapidly declined at first, while the frequencies of ST^c and AR^c increased. However, instead of elimination of the CH^M chromosomes, equilibria have become established at which the frequencies of the competing chromosomes have become stabilized. This is, of course, the outcome which would be expected if the ST^c/CH^M and AR^c/CH^M heterozygotes were heterotic. How are we to reconcile this result with the data (table 1) which suggest that the heterozygotes are less viable than the homozygotes?

The Working Hypotheses

Two working hypotheses may be suggested at this point. *The First Hypothesis.* The heterozygotes which carry the California and the Mexican chromosomes are always heterotic, that is, superior in adaptive value to the homozygotes. This would explain the establishment of the equilibria observed in cages 66 and 67. The inferior viability of the heterozygotes, observed in populations 55 and 56 (table 1), is compatible with this hypothesis. Indeed, the deficient viability may be overbalanced by other

adaptively valuable qualities, which result in a net adaptive superiority of the heterozygotes.

The Second Hypothesis. The original ST^c/CH^M and AR^c/CH^M heterozygotes, obtained in the F_1 generation of the interracial cross, were not heterotic. Hence, a low viability of the heterozygotes was observed in populations 55 and 56. However, heterosis has developed in populations 66 and 67. The emergence of heterosis in these populations may have taken place if some combinations of California and Mexican chromosomes gave rise to heterotic genotypes, while others did not. In the population cages the genotypes that confer high adaptive values on their possessors are selected, and crowd out less well adapted genotypes. Two variants of the second hypothesis are possible; they will be presented in the Discussion.

Testing the Hypotheses

The two working hypotheses outlined above can be tested experimentally. If the first hypothesis is correct, the viability of the heterozygotes ST^c/CH^M and AR^c/CH^M formed in population cages 66 and 67 after the equilibria have become established must still be lower than the viability of the ST^c/ST^c and AR^c/AR^c homozygotes. If the second hypothesis is correct, the heterozygotes may have undergone improvement in cages 66 and 67, and their viabilities may now be higher than those of the homozygotes.

In October, 1950, about 40 pair matings were made from flies hatched in population cage 66. The gene arrangements were determined in 8 larvae from the offspring of each pair. Where necessary, other pair matings were made in the following generations. As a result, 10 strains homozygous for ST^c, and 10 homozygous for CH^M, were established. These new ST^c and CH^M strains were then intercrossed in ordinary culture bottles. On December 27, 1950, approximately 2500 ST^c/CH^M heterozygotes were placed in population cage 75, at 25°C. Among the adult flies hatching in this cage in the next generation, 200 females and 200 males were isolated and outcrossed in individual cultures to known ST/ST homozygotes. Six larvae in the progeny of each culture were examined for gene arrangement. The chromosomal constitution of the 400 parents is shown in table 3.

TABLE 3

THE RELATIVE VIABILITY OF HOMOZYGOTES AND HETEROZYGOTES FOR CALIFORNIA AND MEXICAN CHROMOSOMES AFTER A PROCESS OF NATURAL SELECTION

	Cage 75		
	CH^M/CH^M	ST^c/CH^M	ST^c/ST^c
Observed ♀♀	34	117	49
Viability	0.53 ± .11	1	0.78 ± .14
χ^2	9.95		1.99
Observed ♂♂	35	117	48
Viability	0.55 ± .11	1	0.76 ± .14
χ^2	9.20		2.32

The ST^c/CH^M heterozygotes now have a viability significantly superior to the homozygous ST^c/ST^c and apparently superior to CH^M/CH^M in both sexes. This was not the situation observed in 1948 (table 1). Heterosis has, indeed, developed in population cage 66. The second hypothesis is correct.

MATHEMATICAL ANALYSIS OF SELECTION EXPERIMENTS

If more than the roughest qualitative idea of what is occurring is desired, a mathematical model must be set up, and some attempt made to estimate the relevant parameters. The pertinent mathematical model, and a method of estimating the adaptive values by the method of least squares, have been furnished by Sewall Wright (Wright and Dobzhansky, 1946). The method of Wright, like any other statistical method, has certain limitations. Among these, in the present case, are the lack of an error estimate, the occasional obtaining of biologically impossible negative adaptive values, and the requirement that the adaptive values remain constant during the period under consideration.

In cage 66 the adaptive values have almost certainly changed during the course of the experiment. To meet this need a simple method has been devised which may be used for analyzing such data and which also gives some estimate of the possible range of the true adaptive values. In a later paper, it is hoped to give a more precise investigation of methods for finding adaptive values.

Samples were taken from cages 66 and 67 at varying intervals, each of which was greater than a generation. (The length of a generation under those conditions is 25 days.) These intervals will hence be called periods rather than generations. Table 4 gives the results.

Wright (Wright and Dobzhansky, 1946) has given formulas for the change in $\tilde{q}$ from one generation to the next, where $\tilde{q}$ is the fraction of CH chromosomes in the population. Haldane (1932) has pointed out that formulas for selection take a simpler form in terms of $\tilde{x} = \tilde{q}/(1 - \tilde{q})$, the ratio of CH chromosomes to the other chromosomes in the population. (For some parts of the later analysis it is desirable to take the ratio of the less frequent to the more frequent chromosome, so that $\tilde{x}$ is less than one.) The sample estimate of $\tilde{x}$ is x, the ratio of the number of CH chromosomes to the number of the other chromosomes in a sample. The variance of x is approximately $x(1 + x)^2/n$, where n is the number of chromosomes sampled. In figures 1 and 2, x is plotted against time for cages 66 and 67. The plot is semilogarithmic because the graph should then be closer to a straight line for constant adaptive values. The middle solid line connects values of x, while the other two solid lines connect values of $x \pm 1.96\sigma_x$. The true values of $\tilde{x}$ in the cage might easily lie anywhere within the resulting band, or even somewhat outside of it at a few points. In the long run, 5 per cent of the plotted $x \pm 1.96\,\sigma_x$ will fail to include the true $\tilde{x}$.

We now come to the calculation of adaptive values. We will take the adaptive value of heterozygotes as 1, of CH homozygotes as W_1, and of the

TABLE 4

RESULTS OF SELECTION IN CAGE 66, STARTED WITH CH^M/ST^C HETEROZYGOTES, AND IN CAGE 67, WITH CH^M/AR^C HETEROZYGOTES. EACH SAMPLE CONTAINED 300 CHROMOSOMES. FURTHER EXPLANATION IN THE TEXT.

Period number	Elapsed time in days	Length of period in generations t	Number of CH	Percentage of CH	Ratio of CH to ST or AR x	Standard error σ_x	Adjusted change in x r	Coefficients for finding adaptive values	
								a	b
				Cage 66					
0	0		150	50.0	1.000	0			
1	30	1.20	129	43.0	.754	.063	.791	.265	1.265
2	60	1.20	109	36.3	.571	.050	.792	.508	.952
3	100	1.60	82	27.3	.376	.038	.771	.727	.741
4	140	1.60	70	23.3	.304	.033	.876	.765	.429
5	180	1.60	87	29.0	.408	.040	1.201	.528	.253
6	220	1.60	84	28.0	.389	.039	.970	.623	.421
7	310	3.60	92	30.7	.442	.042	1.036	.576	.375
8	360	2.00	107	35.7	.554	.049	1.120	.451	.395
9	420	2.40	95	31.7	.463	.043	.928	.523	.597
				Cage 67					
0	0		150	50.0	1.000	0			
1	30	1.20	125	41.7	.714	.060	.755	.324	1.324
2	60	1.20	115	38.3	.622	.054	.891	.408	.802
3	100	1.60	96	32.0	.471	.044	.840	.568	.740
4	140	1.60	101	33.7	.507	.046	1.048	.483	.449
5	180	1.60	81	27.0	.388	.039	.845	.676	.601
6	220	1.60	70	23.3	.304	.033	.860	.775	.451
7	310	3.60	74	24.7	.327	.035	1.021	.675	.298
8	360	2.00	72	24.0	.316	.034	.982	.691	.333

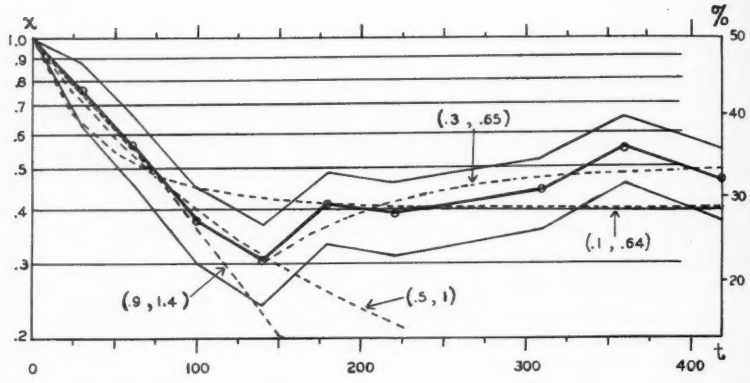

FIGURE 1. Results of cage 66. Vertical axis gives ratio of CH^M to ST^C (on left scale), percentage CH^M (on right scale). Horizontal axis time in days. Circles are sample values, upper and lower solid lines give 95 per cent confidence limits for true values. Dotted lines give theoretical changes if the adaptive values indicated were true.

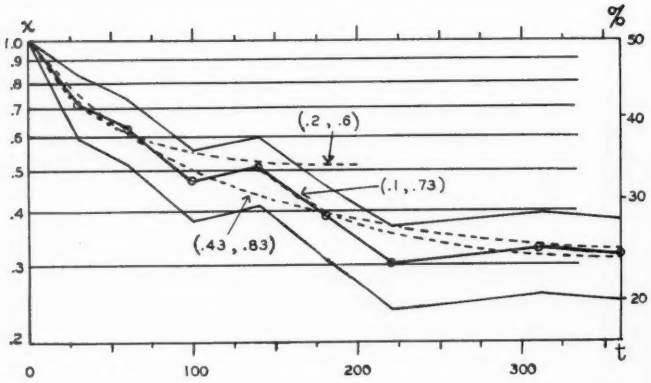

FIGURE 2. Results of cage 67. Vertical axis gives ratio of CH^M to AR^C. Remainder of explanation same as in Fig. 1.

other homozygotes as W_2. If the Hardy-Weinberg equilibrium holds at the egg stage, CH/CH, CH/ST, ST/ST occur in the proportions $\tilde{x}_o^2 : 2\tilde{x}_o : 1$. After selection the proportions become $W_1\tilde{x}_o^2 : 2\tilde{x}_o : W_2$, and in the next generation $\tilde{x}_1 = (W_1\tilde{x}_o^2 + \tilde{x}_o)/(\tilde{x}_o + W_2)$, or

$$\tilde{x}_1/\tilde{x}_o = (W_1\tilde{x}_o + 1)/(\tilde{x}_o + W_2). \tag{1}$$

The ratio $\tilde{x}_1/\tilde{x}_o$ changes more slowly from one generation to the next than does $\tilde{x}$ itself; it is for this reason that a semilog plotting is used in figures 1 and 2. The dotted lines in these figures are the result of applying (1) for certain W's discussed below. If the actual time interval from $\tilde{x}_o$ to $\tilde{x}_1$ is t generations instead of one, equation (1) will no longer serve. If the dotted lines in figures 1 and 2 were actually straight lines it could easily be shown that the correct formula would be

$$\left(\frac{\tilde{x}_1}{\tilde{x}_o}\right)^{1/t} = \frac{W_1\tilde{x}_o + 1}{\tilde{x}_o + W_2} \tag{2}$$

This formula is the best simple approximation to the true one and will be used as the basic formula. Its validity depends on the essential linearity of short portions of the dotted lines. The observed values of the left hand member of (2) are denoted by r in table 4.

Formula (2) can be solved for W_2, giving

$$W_2 = \tilde{a} + \tilde{b}\,W_1, \text{ where}$$
$$\tilde{a} = \left(\frac{\tilde{x}_o}{\tilde{x}_1}\right)^{1/t} - \tilde{x}_o, \text{ and} \tag{3}$$
$$\tilde{b} = \tilde{x}_o\left(\frac{\tilde{x}_o}{\tilde{x}_1}\right)^{1/t}.$$

We thus see that knowledge of what occurs during a single period does not give us the separate values of W_1 and W_2, but only a linear relation between them. Given any two periods, not necessarily consecutive, we obtain two such equations, which can be solved to give W_1 and W_2. This can be done algebraically, or by plotting the two lines and finding the point of intersection. When this is done with actual sample data it often happens that the point of intersection involves a negative value of W_1, W_2 or both. This is biologically impossible, and means that no positive pair of W_1, W_2 could exactly reproduce the observed changes. This discrepancy could be due to either sampling error or an actual change in one or both W's. The lines from a number of periods can be plotted on the same graph, and one can then see by inspection which periods are consistent with each other and what values of W_1 and W_2 are likely.

Examination of figures 1 and 2 suggests that periods 1-4 of cage 66, 5-9 of cage 66, 1-4 of cage 67 and 5-8 of cage 67 are comparable. The corresponding lines from formula (3) are plotted in figures 3-6 and the values of a and b are given in table 4. Before further discussing figures

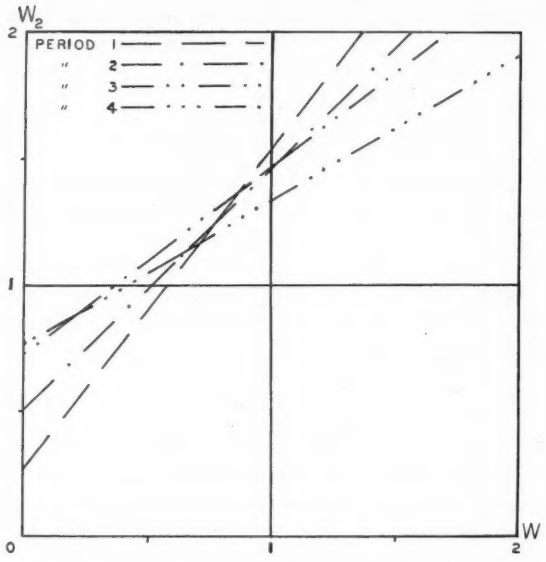

FIGURE 3. Adaptive values for early periods of cage 66. Any pair W_1, W_2 lying on a given line are compatible with the changes during the corresponding period.

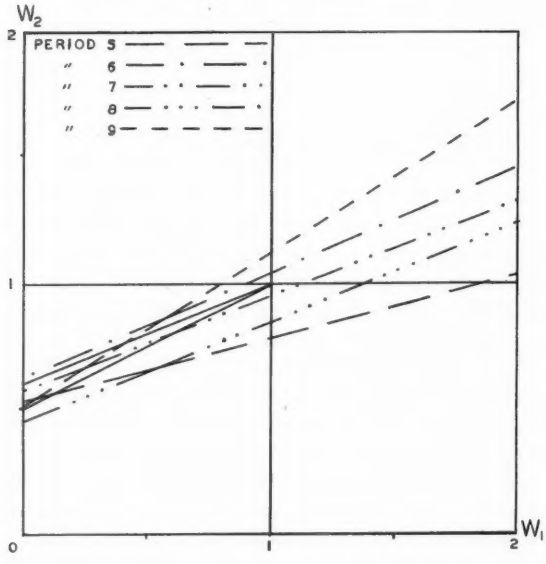

FIGURE 4. Adaptive values for later periods of cage 66. Any pair W_1, W_2 lying on the lower solid line are compatible with equilibrium at $\bar{x} = .5$, any pair on the upper solid line with $\bar{x} = .4$. Interpretation of broken lines same as in Fig. 3.

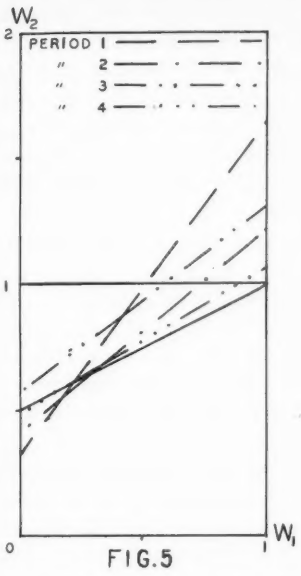

FIG.5

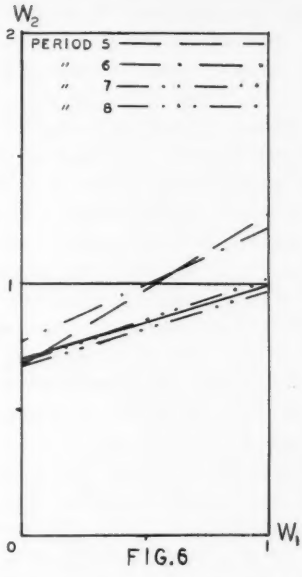

FIG.6

FIGURES 5 AND 6. Adaptive values for cage 67. Interpretation same as in Figs. 3 and 4. Solid line in Fig. 5 for $\bar{x} = .5$, solid line in Fig. 6 for $\bar{x} = .3$.

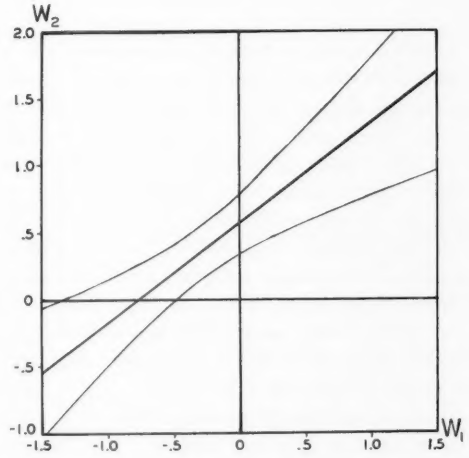

FIGURE 7. Limits of error for line of period 3, cage 67. For interpretation see text.

3–6, the sampling error of these lines will be discussed. Inspection indicates that the line for period 3 in cage 67 is incompatible with the other lines in figure 5 and accordingly this line is chosen for an illustrative example. In figure 7 the line $W_2 = a + bW_1$ corresponding to the observed x's is shown, together with a hyperbola that contains it. If a corresponding figure were drawn for a large number of different samples, the true $\tilde{W}_2 = \tilde{a} + \tilde{b}\tilde{W}_1$ corresponding to the true $\tilde{x}$'s would be contained entirely within the two branches of the hyperbola in 95 per cent of all cases. In our particular figure we can then say that any line drawn in such a way as not to cross either branch of the infinitely extended hyperbola could be the true line at the 95 per cent confidence level. The formula for the hyperbola is too complicated and of insufficient importance to give here, and the calculations are so lengthy that they were only done for this one case; however, all samples contained 300 chromosomes and had roughly the same error, so that the other lines calculated would have an error of the same order of magnitude as in figure 7.

With this indication of the uncertainty of the computed lines, we will proceed to study figures 3–6. Periods 1–3 of cage 66 (fig. 3) intersect in the neighborhood of $W_1 = .9$, $W_2 = 1.4$. (For brevity we will hereafter refer to this as the point (.9, 1.4,) etc. The theoretical series of values of $\tilde{x}$, starting with $\tilde{x} = 1$, resulting from this W_1 and W_2, can be calculated by formula (1), and are plotted as a dotted line in figure 1, taking the interval between generations as 25 days. This theoretical line is in good agreement with the observed for periods 1–3, but thereafter decreases rapidly, ultimately approaching $\tilde{x} = 0$. Period 4 intersects with 1 and 2 near (.5, 1), and the corresponding theoretical line is surprisingly enough in good agreement with the observed for all four periods, although thereafter it also tends toward $\tilde{x} = 0$.

Turning now to periods 5–9, figure 4 shows that many of the lines are nearly parallel and fail to cross, while the observed intersections are at widely separated places. Study of figure 1 shows that this is to be expected, since x fluctuates in an irregular manner. The main conclusion from figure 1 is that x has reached an equilibrium somewhere between .4 and .5 with superimposed chance fluctuations. Equilibrium can only occur with W_1 and W_2 both between 0 and 1; for equilibrium at $\bar{x}$, W_1 and W_2 must lie on the straight line between the points $(0, 1 - \bar{x})$ and (1, 1). The lines corresponding to $\bar{x} = .4$ and $\bar{x} = .5$ are shown as solid lines in figure 4. Considering only periods 5–9, there seems to be an increase from an original value of $x = .3$ to a final equilibrium at approximately .5. From figure 4, the most reasonable W values giving this $\bar{x}$ are (.3, .65). The corresponding theoretical line for $\tilde{x}$ is given in figure 1.

An attempt was also made to fit a single pair of W values to the entire experiment by a somewhat different method. For the entire experiment there seems to be an initial rapid drop followed by fluctuation about an equilibrium at about .4. The line for $\bar{x} = .4$ in figure 4 gives the possible pairs W_1, W_2 that result in this equilibrium. The smaller W_1 is chosen on

this line, the more rapid is the early decline in $\tilde{x}$. Trial and error then gives $W_1 = .1$, $W_2 = .64$ as the pair giving the best over-all fit with the observed values, and the corresponding theoretical line is shown in figure 1. The fit is as good as can be expected, considering the fluctuations observed, but much of the theoretical line lies outside the confidence band. On the other hand the pairs (.5, 1.0) for periods 1–4 and (.3, .65) for periods 5–9, give the theoretical lines which lie wholly within the confidence bands, and it hence seems likely that a change in adaptive values occurred.

While the assumption of constant W's for the first four periods and a different set of constant W's for the remaining periods gives a good fit, this is not to be taken as proof of an abrupt change in adaptive values at the end of the fourth period. A gradual change is much more likely. Unfortunately we do not have enough information to see exactly when the change in W took place, or how long it took. The two sets of W's given above give merely the simplest interpretation of the data.

Turning now to cage 67, the first four periods in figure 2 seem to be leading to an equilibrium at $\bar{x} = .5$. In figure 5 the lines for periods 1, 2 and 4, and for $\bar{x} = .5$ intersect near (.2, .6) and the corresponding theoretical $\tilde{x}$'s are in good agreement with the first four periods. The line for period 3 in figure 5 is apparently incompatible with (.2, .6) but figure 7, already discussed, shows that it is quite possible for the true line for period 3 to pass through this point. During periods 5 to 8 a further drop occurs, to an apparent equilibrium near $\bar{x} = .3$. Figure 6 contains the lines for these periods and this $\bar{x}$. Periods 7, 8 and $\bar{x} = .3$ are compatible with any W_1 from 0 to 1 and any W_2 from .7 to 1. Period 5 suggests a low value of W_1 and hence the pair (.1, .73) was tried. The corresponding theoretical values of $\tilde{x}$, starting with $\tilde{x} = .5$ at the beginning of period 5, give an excellent fit. Again for this cage an attempt was made to find a single pair of W's that would serve for the whole experiment. Periods 1, 2, 7, 8, and $\bar{x} = .3$ all intersect near (.43, .83). The resulting theoretical $\tilde{x}$ line lies almost wholly within the confidence band, and hence, for this cage, the results can be accounted for by a constant pair of adaptive values, although naturally a better fit is obtained by using one pair for the early part and another pair for the later part of the experiment.

MATHEMATICAL DISCUSSION OF VIABILITY EXPERIMENTS

We turn now to the viability experiments. Suppose eggs are laid in a cage in the proportions 1 CH/CH : 2 CH/ST : 1 ST/ST. After selection the adults are classified as to genotype. There is a certain probability of misclassification; suppose the combination of selection and misclassification results in probabilities α, β, and γ that an adult be classified as CH/CH, CH/ST, and ST/ST and that the numbers actually recorded are a, b, and c. Suppose the viabilities of the three types be taken as $\tilde{V}_1$, 1 and $\tilde{V}_2$. (The letter V is used since the viability V is only one component of the adaptive value W discussed above.) Then the initial proportions 1 : 2 : 1 become $\tilde{V}_1 : 2 : \tilde{V}_2$ after selection. If six larvae are examined in classifying each

adult, there is probability 1/64 that a given heterozygote will be classified CH/CH, and 1/64 that it will be classified ST/ST. Hence the probabilities of being listed in each of the three classes α, β and γ become $(32\,V_1 + 1)/d$, $62/d$, $(32\,V_2 + 1)/d$, where $d = 32(V_1 + V_2 + 2)$. In the special case of equal viabilities, $V_1 = V_2 = 1$, and $\alpha = \gamma = 33/128$, $\beta = 62/128$.

If sampling is continued until a fixed number b of heterozygotes have been obtained, $\underline{a}$ will have a Pascal distribution, with mean $b\alpha/\beta$ and variance $b\alpha(\alpha + \beta)/\beta^2$. [The Pascal distribution is discussed in section 11.3 of an excellent new book on probability and its applications to genetics, (Feller, 1950)]. To test the hypothesis that $\hat{V}_1 = 1$, we then have for large samples

$$\chi^2_L = \frac{(a - b\alpha/\beta)^2}{\sigma^2_a} = \frac{(62a - 33b)^2}{3135b}, \tag{4}$$

with χ^2_L distributed as χ^2 with one degree of freedom. Dobzhansky (1950) used a formula for χ^2 which was supplied by his present coauthor, who assumed, without investigation of the matter, that $\underline{a}$ would have a Poisson distribution. That formula was erroneous, all computed χ^2's being too small by a factor of $62/95 = .6526$. Luckily this error does not affect the conclusions reached by Dobzhansky, all but one of his "significant" chi squares remaining significant.

If, on the other hand, sampling is continued until a fixed number of flies have been classified, for the study of $\hat{V}_1$ we can proceed as if $\underline{a}$ comes from a binomial sample of a + b observations, with the probability of CH/CH being $(32\,\hat{V}_1 + 1)/(32\,\hat{V}_1 + 63)$. We then have the statistic

$$\chi^2_D = \frac{(a - Ea)^2}{Ea} + \frac{(b - Eb)^2}{Eb} = \frac{(62a - 33b)^2}{2046\,(a + b)} \tag{5}$$

This statistic was suggested by Dr. Everett R. Dempster, who pointed out that the formula in Dobzhansky (1950) was incorrect. Continuing our analysis for the binomial case, setting $\underline{a}$ equal to its expected value $(a + b)(32\,\hat{V}_1 + 1)/(32\,\hat{V}_1 + 63)$ and solving for $\hat{V}_1$, we obtain the sample estimate

$$V_1 = \frac{62a}{32b} - \frac{1}{32}, \tag{6}$$

which is identical with the estimate in Dobzhansky (1950). Thus the two assumptions of constant b or constant a + b lead to the same estimate V_1. The two assumptions lead to quite different variances for a; however V_1 depends only on a/b, and this ratio has the same large sample variance in the two cases. The true variances depend on the unknown probabilities α, β and γ, but in practice they must be replaced by the sample values a, b and c, and this is valid in large samples. It can be shown that, under either mode of sampling, the sample estimate V_1 is approximately normally distributed with mean $\hat{V}_1$ and variance

$$\sigma^2_{V_1} \cong \frac{(32V_1 + 1)(32V_1 + 63)}{(32)^2 b} \cong \left[\frac{62}{32}\right]^2 \left[\frac{a(a + b)}{b^3}\right] \tag{7}$$

We can now propose two additional chi square formulas, $\chi^2_V = (V_1 - 1)^2/\sigma^2_{V_1}$ and $\chi^2_a = [a - 33\ (a + b)/95]^2/[ab/(a + b)]$. All four of these formulas are valid in very large samples and would give equivalent results. However, in moderate sized samples they give different results. As an example, in cage 55 (table 1) for ST/ST females, where V is greater than one (V = 1.866), we find $\chi^2_V = 5.0$, $\chi^2_a = 8.2$, $\chi^2_D = 9.1$, $\chi^2_L = 11.8$. On the other hand in cage 75 (table 3) for CH/CH males, where V is less than one (V = .548), the relationships are reversed, and $\chi^2_V = 16.2$, $\chi^2_a = 11.7$, $\chi^2_D = 9.2$ and $\chi^2_L = 7.8$. The relative order of magnitude of the four chi squares should be the same as this in general, reversing as V is greater or less than one. All these chi squares are approximations valid in large samples; χ^2_V and χ^2_a should require larger samples to be satisfactory than do the other two, hence one of the other two should be used. It would probably be legitimate to use χ^2_D for $V < 1$ and χ^2_L for $V > 1$; however, this raises certain unsolved problems, and for the present χ^2_D is recommended, as a compromise for routine use. Values of χ^2_D for the present data are given in tables 1 and 3. The statistic χ^2_D for V_1 and the corresponding χ^2_D statistic for V_2 are logically independent, since the viability of one homozygote relative to the heterozygote need not affect the viability of the other heterozygote. On the other hand the two chi-squares are not independent statistically; *i.e.* the sum of the two may not be taken as a chi-square with two degrees of freedom. This comment applies to the other chi-squares discussed above.

There is another aspect to the use of χ^2_V. This amounts to using V with its approximate standard error from (7). This has the great advantage that we can test the hypothesis that $\tilde{V}$ has any given value. Furthermore an approximate confidence interval for $\tilde{V}$ can be given in the usual way as $V \pm C\sigma_V$, where C is 1.96, 2, 3, etc., as desired. These confidence intervals serve for most practical purposes. However, if greater accuracy is desired, particularly in small samples or when V is far from 1, we can consider $\underline{a}$ as a binomial variable from a sample of a + b observations. Then upper and lower confidence limits may be obtained for the expected value of $\underline{a}$ (see e.g. Fisher and Yates, 1943) and these two values of $\underline{a}$ substituted into (6) to obtain more accurate confidence limits for $\tilde{V}$. As a rule the length of the confidence intervals obtained in these two ways are about the same, but the limits by the more accurate method are both somewhat higher. For example, in the two cases used in the previous paragraph the standard error gives $(.33 < \tilde{V} < .77)$ and $(1.10 < \tilde{V} < 2.63)$ with 95 per cent confidence coefficient, while the more precise method gives $(.35 < \tilde{V} < .79)$ and $(1.23 < \tilde{V} < 2.82)$. Tables 1 and 3 give the values of V and their standard errors for the present data.

CONCLUSIONS FROM THE STATISTICAL ANALYSIS

We now turn to a consideration of what actually happened in cages 66 and 67. The strains used in cage 66 were tested for viability in cage 55 before cage 66 was started, and strains recovered from cage 66 during period 8 were tested for viability in cage 75. Initially, in cage 55, $V_1 = .98 \pm .25$ and $V_2 = 1.87 \pm .39$ for females, while for males $V_1 = .81 \pm .18$

and $V_2 = .86 \pm .18$. There was a significant difference between males and females. Assuming that adaptive value depends only on viability and depends equally on males and females, we would guess the adaptive values as $W_1 = .87$ and $W_2 = 1.26$. Periods 1–3 of cage 66 gave estimates $W_1 = .9$, $W_2 = 1.4$ which are in excellent agreement. During the later periods of cage 66 we estimated the adaptive values as $W_1 = .3$, $W_2 = .7$ with considerable margin for error. The flies derived from this cage gave viabilities of about $V_1 = .54$ and $V_2 = .77$, essentially the same for males and females. Again the agreement is fairly good, considering the variability of both the W's and V's. It seems almost certain that both the adaptive value and viability of ST homozygotes declined relative to the heterozygotes, and there is some indication that the same happened for CH homozygotes. The adaptive values and viabilities are in fairly good agreement.

The situation is somewhat different for cage 67. The course of events here could be explained either by adaptive values (.2, .6) for the early period and (.1, .73) for the later period, or by constant adaptive values (.43, .83). Viability was tested only before the selection experiment, so we cannot see whether the viability changed. In cage 56 the viability values were $V_1 = 1.1$, $V_2 = 1.9$ for females and $V_1 = 1.2$, $V_2 = 1.4$ for males, with an average value for both sexes of $V_1 = 1.2$, $V_2 = 1.5$. Study of figure 5 shows that these values, taken as adaptive values, would be compatible with periods 2 and 3, or even with periods 1, 2 and 3 remembering the variability shown in figure 7, in spite of the tremendous deviation from the best guess from figure 5. Hence in this cage also we might suppose adaptive values and viability are nearly equal, and have changed during the course of the experiment, but it seems more likely that adaptive values and viability are quite different; and the adaptive value may have remained unchanged, although it might equally well have changed a good deal.[3]

DISCUSSION

The development of heterosis may be visualized in two ways. First, it may be due to selection of coadapted third chromosomes. Secondly, it may involve selection of whole new genotypes. It should be kept in mind that the ancestral population of cage 66 included 15 different California and 12 different Mexican strains. Therefore, at least 15 different ST^c and 12 different CH^m chromosomes were involved, and probably considerably more since each "strain" may have carried up to four or more different chromosomes (two descended from the wild female progenitor and two or more from the male or males with which she mated before capture). It is possible, then, that some of the California chromosomes introduced into cage 66, or formed there by crossing over, gave heterosis with some of the CH chromosomes from Mexico. Such "heterotic" chromosomes would then

[3] No account has been taken in this analysis of the possibility of genetic drift. The size of the cage populations is much larger than the sample size, but there may still be at least some error due to drift. It is hoped to consider this aspect in a later paper.

be multiplied by selection, at the expense of the chromosomes which gave poorly viable heterozygotes. On the other hand, it is possible that heterosis arises through interaction between the genes located in the third chromosomes and genes in other chromosomes. In other words, the heterosis exhibited by the ST/AR, ST/CH, and AR/CH heterozygotes in the California populations may be the property of the genotype of the California race. Now, countless new genotypes were doubtless formed in the cages 66 and 67 by recombination of the California and the Mexican genotypes. Among these recombination products, some of the ST^{c}/CH^{M} heterozygotes which also carried certain constellations of the California and Mexican gene blocks, may have exhibited superior adaptive values. Such adaptive genotypes would be multiplied and established by natural selection.

The data at hand do not permit discrimination between these possible mechanisms of the development of heterosis. It may, nevertheless, be pointed out that the second mechanism would entail selection in our experiments of previously non-existent but adaptively coherent genotypes, which might differ both from the California and from the Mexican races in many genes. The greater the number of the gene differences involved, the less frequent will be the origin of the new genotype by recombination, and, consequently, the less easily repeatable will become the process. Evolutionary changes become less and less easily repeatable as they involve more and more gene substitutions in adaptively integrated genotypes.

SUMMARY

Hybrids of the California and the Mexican races of *Drosophila pseudoobscura* were kept in population cages for approximately 15 generations. A preliminary study of viability, and the early course of the population in the cages, showed no heterosis for the heterozygotes which carried a California and a Mexican third chromosome. Heterosis has, however, developed during the experiment, as indicated by the attainment of equilibrium and by a study of the viability of flies derived from the cage. Both tests gave statistically significant results. Methods were developed for estimating adaptive values and viability in such experiments, and for investigating the possible range of values of these constants consistent with the sampling error involved.

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LITERATURE CITED

Da Cunha, A. Brito, 1949, Genetic analysis of the polymorphism of color pattern in *Drosophila polymorpha*. Evolution 3: 239–251.

Dobzhansky, Th., 1947a, Adaptive changes induced by natural selection in wild populations of Drosophila. Evolution 1: 1–16.

1947b, Genetics of natural populations. XIV. A response of certain gene arrangements in the third chromosome of *Drosophila pseudoobscura* to natural selection. Genetics 32: 142–160.

1948, Genetics of natural populations. XVIII. Experiments on chromosomes of *Drosophila pseudoobscura* from different geographical regions. Genetics 33: 588–602.

1949, Observations and experiments on natural selection in Drosophila. Proc. 8th Int. Congr. Genetics 210–224.

1950. Genetics of natural populations. XIX. Origin of heterosis through natural selection in populations of *Drosophila pseudoobscura*. Genetics 35: 288–302.

Dobzhansky, Th., and Howard Levene, 1948. Genetics of natural populations. XVII. Proof of operation of natural selection in wild populations of *Drosophila pseudoobscura*. Genetics 33: 537–547.

Feller, W., 1950. Probability theory and its applications. New York.

Fisher, R. A., and F. Yates, 1943. Statistical tables for biological, agricultural and medical research. London.

Haldane, J. B. S., 1932. The causes of evolution. London.

Wallace, B., 1948. Studies on "sex-ratio" in *Drosophila pseudoobscura*. I. Selection and "sex-ratio." Evolution 2: 189–217.

Wright, Sewall, and Th. Dobzhansky, 1946. Genetics of natural populations. XII. Experimental reproduction of some of the changes caused by natural selection in certain populations of *Drosophila pseudoobscura*. Genetics 31: 125–156.

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PYLE-BLACK PLUMAGE IN THE FOWL

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Sexually dimorphic and dichromatic zoning of red-gold pigmentation is manifested in plumage of the Red Pyle Game. It occurs on a colorless (white) background due to inhibition of black by gene *I* (dominant white). The Red Junglefowl, Black-breasted Red Game, and Brown Leghorn illustrate pyle-zoning on a black background. Breeding experiments indicate that pyle-zoning is attributable to partial restriction of a specific black pigment by gene e^+, the wild type allele at the *E* (extended black) locus (*cf.* Smyth and Bohren, 1949). Expression of pyle-zoning is complete, fractionated, reduced, or absent (phenocryptic) dependent upon modifying polygenic complements.

Stippling is associated with an autosomal gene *(Sg)* which restricts black within feathers of juvenile plumage in both sexes; with the exception of certain feather groups in males of some Red Junglefowl biotypes and of Light Brown Leghorns, expression of stippling is limited to female adult plumage. Genotype in wild type plumage is conceived to be $(e^+\ e^+\ Sg\ Sg)$. Segregants of Brown Leghorn ♂ $(e^+\ e^+\ Sg\ Sg)$ × White Minorca ♀ $(E\ E\ sg\ sg)$, and of Golden Campine ♂ $(e^+\ e^+\ sg\ sg\ Bg\ Bg)$ × Brown Leghorn ♀ $(e^+\ e^+\ Sg\ Sg\ bg\ bg)$, have been isolated and studied. Such fowls carry pyle-zoned black plumage in both sexes, incident to the genotype $(e^+\ e^+\ sg\ sg\ bg\ bg)$. Black in these birds is comparable to recessive black mammalian pelage, and has been reported previously by Goodale (1926), and by Serebrovsky (1926).

Pyle-black ♂ × Brown Leghorn ♀ produced F_1 progeny with males indistinguishable from the leghorn phenotype, and females characterized by incomplete, apico-marginal patterning of stippled feathers. Stippling *(Sg)* and autosomal barring *(Bg)* have been found epistatic to black pigment of pyle-black $(e^+\ e^+)$ plumage; whereas, both secondary pattern genes are hypostatic to black pigment of fowls carrying extended black *(E E)* plumage, *e.g.*, Black Minorca and Black Langshan. A similar situation prevails for penciling *(Pg)*, *e.g.*, Partridge Rock and Dark Cornish, as suggested by studies now in progress.

Genetically and physiologically distinct forms of black pigment coincide with the genotypes $(e^+\ e^+)$ and *(E E)*. Wild type and immediately derived plumage patterns (involving mutated secondary pattern factors) thus far tested are genetically $(e^+\ e^+)$. Complete or modified pyle-zoning occurs in all junglefowls, associated with secondary patterns such as stippling, lacing, or barring. Examination of junglefowl and hybrid junglefowl skins in the American Museum of Natural History collection was made possible through the courtesy of Mr. Jean Delacour. Phenotypes observed were

compatible with the view that all junglefowls are of the genotype (e^+ e^+). It appears that recognition of wild type (e^+) black is fundamental in significance for plumage genetics, classification of plumage patterns, and for identification of secondary pattern factor alleles as dominant or recessive. Wild type (e^+) black would seem to be the rational standard of reference, rather than extended (E) black in mutated domestic forms of fowl.

LITERATURE CITED

Goodale, H. D., 1926, Salmon blacks. Anat. Record 34: 171–172.

Serebrovsky, A. S., 1926, Genetics of the domestic fowl (Russian). Mem. Anikowo Genet. Sta. near Moscow, pp. 1–136 (Abstracted by L. C. Dunn, J. Hered. 19: 511–519, 1928).

Smyth, J. R., Jr., and B. B. Bohren, 1949, A multiple allelic series affecting feather color in the domestic fowl. Poultry Sci. 28: 782 (Abstr.).

LETTERS TO THE EDITORS

Correspondents alone are responsible for statements and opinions expressed. Letters are dated when received in the editorial office.

SECONDARY SEX RATIO, BODY WEIGHT AND LENGTH AND MORTALITY RATE AMONG INFANTS

A study of birth weight and mortality rate among infants is of great importance, especially in Egypt where little or no extensive analysis has been carried out along this line. All single babies who were born in one of the maternity hospitals for poor classes in Cairo during the year 1949 were included. The secondary sex ratio was expressed as the number of males per 100 females born. The mortality rate was calculated by taking the percentage of babies who died within a week after birth to the total number of babies born. The data included 1762 births, of whom 930 were boys and 832 were girls.

The secondary sex ratio was found to be 111.8. This comparatively high sex ratio is in agreement with Marshall[1] who reported that poor classes of people have a higher sex ratio than the average. In accordance with Crew[2] sex ratio was lower in the cool months (105.2) than in the warm months of the year (120.8).

For all babies studied, the mean birth weight and the mean body length were 6.8 lbs. and 19.1 inches, respectively. On the average, boys weighed 0.2 lb. more than girls at birth, thus agreeing with Donald.[3] The babies who died within a week after birth weighed on the average 2.0 lbs. less and were 2.2 inches shorter in body length than those who survived. Summer babies were slightly lighter and shorter than the average.

The mortality rate averaged 12.6 per cent. Undernourishment and premature birth are believed to be the main causes for such a high rate of mortality. Spring had the highest rate of mortality (17.9 per cent), while the fall had the lowest one (8.9 per cent).

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LITERATURE CITED

1. Marshall, F. H. A., The Physiology of Reproduction. Longmans Green and Co., London and New York, p. 687, 1922.
2. Crew, F. A. E., Animal Genetics. Oliver and Boyd, Edinburgh and London, pp. 254, 256, 1925.
3. Donald, H. P., Proc. Roy. Soc. Edinburgh, pp. 94, 95, 1939.

GENE-FREQUENCIES AND MENDELIAN RATIOS

In 1908, Hardy and Weinberg independently stated a basic principle of population genetics which has since been discussed in a number of elementary textbooks of genetics[1, 2, 3, 4, 5] (Sturtevant and Beadle, 1939; Snyder, 1940; Riley, 1948; Stern, 1949; Sinnott, Dunn, and Dobzhansky, 1950), and which has been well explained by Stern.[6]

According to this theory, in a sexually-reproducing population which reproduces by cross-fertilization, the frequency of two alleles will reach an equilibrium in one generation and will tend to remain unchanged from one generation to another so long as there is random mating, no tendency towards a differential survival of the various types and no mutations and provided that the population is a large one and there are no additions to it from the outside once it has arisen. The proportions of the different phenotypes will depend on the relative frequencies of the two alleles and therefore a dominant allele might be present with so low a frequency that recessive phenotypes may considerably outnumber the dominants. It follows, then, that the dominant or recessive nature of a particular allele cannot be determined from the relative frequencies of the various phenotypes. This principle has been recognized for a long time but has not been universally understood nor appreciated, and occasionally an attempt is made to interpret population ratios in terms of a factorial analysis. This procedure is especially tempting if the population ratio happens to simulate a Mendelian or a modified Mendelian ratio.

One of the more recent population studies which have been interpreted in terms of Mendelian ratios is Schütte's[7] (1949) analysis of flower color in *Romulea bulbocodioides,* a South African member of the Iridaceae. In his introduction, Schütte states, "Occasionally colour differences have been used to separate very similar plants into two species differing only in colour. Yet colour differences are often of a purely genetical nature and follow the ordinary Mendelian Laws of Inheritance. Where colouration is due to simple genetical factors, it is sometimes possible to observe this from ordinary field observations." Studying plants on Rondebosch Common in Cape Town, he counted all the plants in a number of areas each a measured square meter starting with random sampling, but later observing only "hybrid areas."

Eleven random samples yielded areas varying from 36 yellow and 31 white to 10 yellow and 55 white with three approaching a ratio of 9 yellow: 7 white. These random areas showed numerous large patches of either yellow- or white-flowered plants and were therefore considered to result largely from inbreeding. Twenty areas which were believed to contain only plants which had arisen by cross-fertilization yielded 392 yellow and 344 non-yellow plants, or 53 per cent yellows; all but four of these were considered as indicating "a dihybrid ratio" (*i.e.,* 9:7) although no statistical constants were given. Schütte suggests that more homozygous types

would have been expected and that their infrequency must indicate very intensive cross-pollination.

The interpretation given for these results is that yellow is dominant over non-yellow. The non-yellows include white, cream, and pale-yellow types. It is suggested that the pale yellows and creams are heterozygotes but no frequencies are given for the various non-yellow types in the twenty "hybrid areas."

If the Hardy-Weinberg law is applied, a ratio of 9 yellow : 7 non-yellow could be approximated even if yellow is recessive to non-yellow provided that the frequencies of the yellow and non-yellow genes are respectively 0.73 and 0.27. With this gene frequency, the population should consist of 0.53 yellows, the homozygous recessive, 0.39 heterozygotes, and 0.07 homozygous whites. If the last two classes form Schütte's non-yellows, the ratio should be 0.53 yellow : 0.47 non-yellow, which is very close to the ratio from his Table 2. In other words, his observed ratios could be obtained even if yellow is recessive to white and they do not prove that yellow is dominant as he assumed. Since he did not tabulate the pale-yellow and white types separately in Table 2, there is no evidence as to how close his ratio comes to the calculated if, as he suggests, the pale yellows are heterozygotes.

Although the Hardy-Weinberg Law may not be strictly applicable to Romulea since there is no evidence that this plant does not reproduce in part by self-fertilization and since there may be some vegetative reproduction from bulbs, the possibility that ratios like Schütte's can be obtained on hypotheses other than his shows that it is dangerous to assume the dominance of a given gene from studies of the frequencies of various phenotypes in a natural population when only two phenotypes can be distinguished.

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LITERATURE CITED

1. Sturtevant, A. H. and G. W. Beadle, 1939, An introduction to genetics. Philadelphia: W. B. Saunders Co.
2. Snyder, L. H., 1940, The principles of heredity. 2nd ed. New York: D. C. Heath and Co.
3. Riley, H. P., 1948, An introduction to genetics and cytogenetics. xii + 596 pp. New York: John Wiley and Sons, Inc.
4. Stem, C., 1949, Principles of human genetics. San Francisco: W. H. Freeman and Co.
5. Sinnott, E. W., L. C. Dunn, and Th. Dobzhansky, 1950, Principles of genetics. 4th ed. New York. McGraw-Hill Book Co.
6. Stem, C., 1943, The Hardy-Weinberg law. Science 97: 137–138.
7. Schütte, K. H., 1949, Observations on colour inheritance in *Romulea bulbocodioides*. Jour. South Afr. Bot. 15: 97–100.

THE HOMOLOGY OF THE "NOTOCHORD" FOUND IN PTEROBRANCHS AND ENTEROPNEUSTS

While examining sections of the curious pterobranch *Atubaria heterolopha* Sato, a suspicion occurred to the writer as to the homology of the "stomochord"—the so-called notochord. This structure is a dorsal diverticulum of the foregut, ending near the brain. A similar structure is found in the enteropneusts. Bateson (1884) was the first to homologize this with the notochord of the vertebrates. He based this idea mainly on the fact that it is a dorsal outgrowth of the digestive tract, and the component cells are vacuolated. This view was accepted by most subsequent authors, and gave rise to the name of "Hemichorda" or "Adelochorda" to designate the group to which these animals belong. A few authors, however, disagreed with this view. Spengel (1893), for instance, supposed that the structure in question is merely a pre-oral part of the gut.

To the writer the "stomochord" seems to be rather a homologue of the anterior lobe of the hypophysis in vertebrates, because it is a diverticulum of the anterior end of the digestive tract, and its blind end is closely apposed to the cerebral ganglion in pterobranchs. It may be objected that, while the stomochord is believed to be derived from the endoderm, the anterior lobe of the hypophysis is of ectodermal origin. But, there is a possibility that the stomochord is ectodermal in origin at least partially (cf. Spengel, 1893). This point would merit a crucial reexamination of adequate material.

The notochord in vertebrates, as well as in cephalochords, arises from the dorsal wall of the archenteron throughout nearly its whole length, and becomes independent of the latter in an early stage of development. The notochord in urochords also develops from endodermal cells intimately related to those giving rise to the digestive tract. It is, however, restricted to the posterior region of the body, without any relation to the brain. Thus, the notochord in all these chordates shows a sharp distinction from the stomochord in pterobranchs and enteropneusts.

The subneural gland of urochords has been homologized with the hypophysis. This structure originates as a part of the brain vesicle of the larva, and becomes isolated from the latter, and acquires a duct opening outside or in the pharynx. This mode of origin reminds us of the posterior lobe of the hypophysis in vertebrates. Possibly, the duct is the homologue of the stomochord in pterobranchs and enteropneusts. At any rate, it seems more natural to homologize the stomochord with the hypophysis, particularly its anterior lobe, rather than with the notochord, in vertebrates.

LITERATURE CITED

Bateson, W., 1884, Note on later stages in the development of *Balanoglossus kowalevskii* (Agassiz), and on the affinities of the Enteropneusta. Proc. Roy. Soc. London 38: 23–30.

Harmer, S. F., 1905, The Pterobranchia of the Siboga Expedition.
Komai, T., 1949, Internal structure of the pterobranch *Atubaria heterolopha* Sato, with an appendix on the homology of the "notochord." Proc. Japan Acad. 25: 19–24.
Sato, T., 1936, Vorläufige Mitteilung über *Atubaria heterolopha* gen. nov. sp. nov., einen in freiem Zustand aufgefundenen Pterobranchier aus dem Stillen Ocean. Zool. Anz. 115: 97–106.
Schepotieff, A., 1907, 1908, Die Pterobranchier. Zool. Jahrb. Anat. Ont. 23: 463–534, 25: 405–494.
Spengel, J. W., 1893, Fauna und Flora des Golfes von Neapel. Enteropneusten.

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Allan, Joyce, 1950. Australian Shells. 470 p. 60 shillings. Georgian House, Melbourne, Australia.

Boletin del Instituto Nacional de Investigaciones Agronomicas, Vol. 10. No. 23, Madrid, Spain.

Botanichesky Zhurnal, Vol. 35, Nos. 2, 3, 4, Academy of Sciences, U. S. S. R.

Gardner, Victor R., 1951. Basic Horticulture. 465 p. $4.75. The Macmillan Co., New York.

Goodey, T., 1951. Soil and Freshwater Nematodes. 389 p. $7.00. John Wiley and Sons, Inc., New York.

Gregory, William K., 1951. Evolution Emerging. Vol. 1, 736 p. Vol. 2, 1013 p. $20.00. The Macmillan Co., New York.

Hudson, W. H., 1951. Letters on the Ornithology of Buenos Ayres. 93 p. $2.75. Cornell University Press, Ithaca, New York.

Mitteilungen des Naturwissenschaftliches Museums der Stadt Aschaffenburg, 1950. 4. Heft der neuen Folge.

Nachrichten der Sammelstelle für Schmarotzerbestimmung, Nr. XXIX, Dezember, 1950. Naturwissenschaftliches Museum der Stadt Aschaffenburg.

Priroda (Nature). Nos. 2-10. 1950. Academy of Sciences, U. S. S. R.

White, M. J. D., 1950. Cytological Studies on Gall Midges. 80 p. $1.00. Univ. of Texas Publication No. 5007, Austin, Texas.